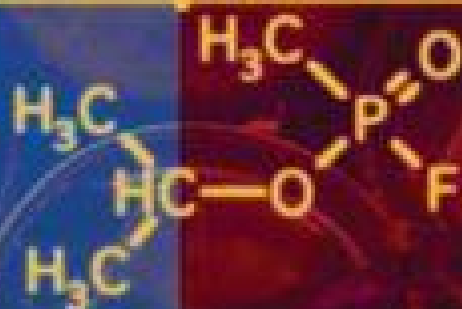


CHEMICAL WEAPONS CONVENTION CHEMICALS ANALYSIS

**Sample Collection,
Preparation
and Analytical Methods**

Editor
Markku Mesilaakso

 WILEY



CHEMICAL WEAPONS CONVENTION CHEMICALS ANALYSIS

Sample Collection, Preparation and Analytical Methods

Edited by

Markku Mesilaakso

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Dedication

To my wife, children, and parents.

About the Editor



Markku Mesilaakso was born in 1957 in Tornio, Finland. His main area of study was structural chemistry and he achieved the highest grade in organic chemistry at the University of Oulu. In 1985, he joined Professor Erkki Rahkamaa's research group and began work using NMR spectroscopy in toxin analysis. In 1992 in Helsinki, he began work in the CW Project of the Ministry for Foreign Affairs, and with Professor Marjatta Rautio he further developed the methods for NMR analysis of CW agents and related chemicals. He received his Ph.D. on this topic. Since the mid 1980s Dr. Mesilaakso has coauthored books in the series *Methodology and Instrumentation for Sampling and Analysis in the Verification of Chemical Disarmament* (the so-called Finnish Blue Books). His scientific publications deal with the analysis of NMR spectral parameters of CW

agents and toxins, and the use of NMR spectroscopy as a complementary method for analysis of Chemical Weapons Convention (CWC) chemicals from the environment. His interests encompass verification and implementation of the CWC, environmental analysis, quality assurance, and training.

Dr. Mesilaakso is currently Acting Director of the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN; former CW Project) at the University of Helsinki. Before this, he worked at VERIFIN as a Research Scientist, Quality Manager and Research Director. He is a member of the Finnish National Authority of the CWC, a member of the Scientific Advisory Board for the Defense of Finland, and a member of the OPCW Validation Group for data evaluation to the OPCW Central Analytical Database.

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Preface

The States Parties to the Chemical Weapons Convention (CWC) have established the Organization for the Prohibition of Chemical Weapons (OPCW) in order to achieve the object and purpose of the Convention. It aims to ensure the implementation of the CWC's provisions, including those for international verification of compliance with it, and to provide a forum for consultation and cooperation among States Parties. The aim of this book is to give a comprehensive view of how to internationally verify compliance with the CWC, in principle, using analytical chemistry and related strategies and methods.

There are currently eighteen analytical laboratories that have established capability, and obtained recognized competence in, the analysis of samples for CWC-related chemicals; these laboratories are the OPCW designated laboratories. The majority of the chapters in this book discuss the analytical methods used in these off-site laboratories. The methods discussed are for the identification of target chemicals from environmental and human origin samples.

The procedures and strategies for on-site sampling and analysis are also discussed. In connection with the verification activities of the OPCW on-site, samples may be taken, for example, during a facility inspection for subsequent on-site analysis. The possibility to send the samples for analysis off-site also exists.

This book aims to serve different readers from the fields of environmental and analytical chemistry as well as researchers in civil and military laboratories. The National Authorities of the States Parties to the CWC may find the book useful as

it covers developments in the area of CWC chemicals analysis. The approaches are practical, and the contributions are particularly useful for those people responsible for developing their own laboratory analysis methods and work instructions.

The book discusses a variety of topics. These include: the requirements of the CWC for verification; sampling; the mobile laboratory and its equipment and software; sample preparation from environmental samples; official inter-laboratory proficiency tests arranged by the OPCW; designated laboratories; the OPCW Central Analytical Database (OCAD); instrumentation, monitoring of hazardous chemicals, analysis strategies, quality assurance and screening; analytical methods including gas and liquid chromatography hyphenated with mass spectrometry or other detectors, nuclear magnetic resonance spectroscopy, infrared spectroscopy, capillary electrophoresis, and the detection of exposure to toxic scheduled chemicals.

I hope that this book will be of great benefit to people working in the field of the verification and implementation of the CWC. Without the professionals who have contributed to this book, it would not have been possible. I therefore express my sincerest thanks to all of the experts who have given their time and effort in publishing their precious knowledge in the field of CWC chemicals analysis.

Markku Mesilaakso

Helsinki, March 2005

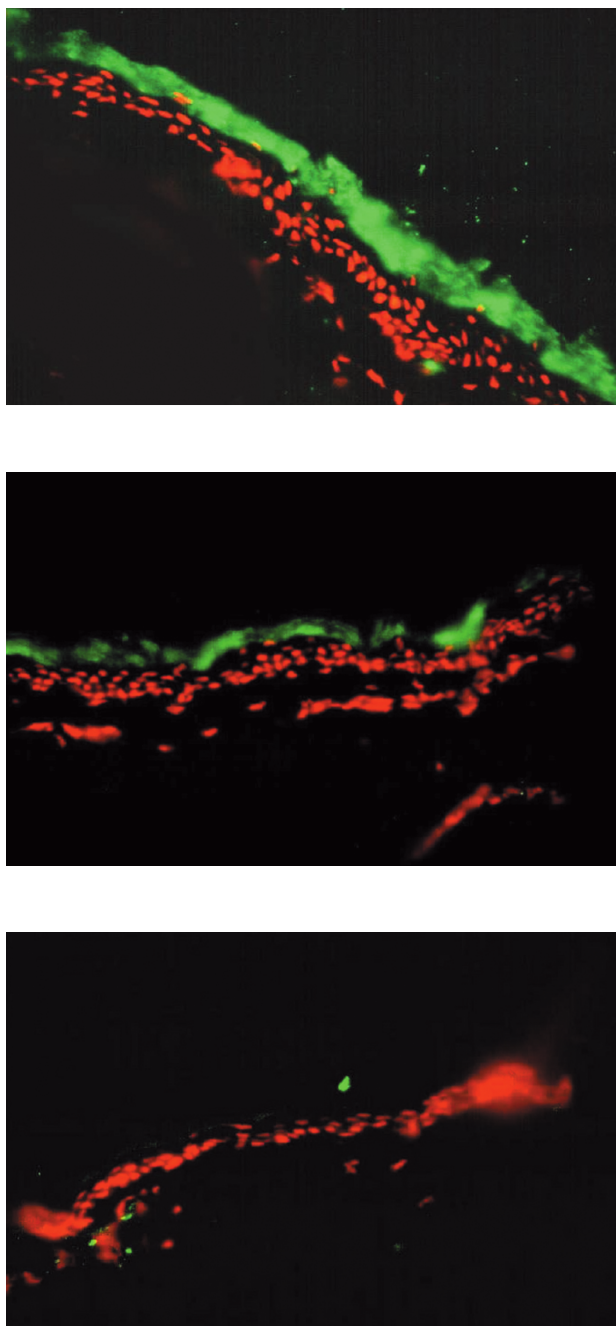


Plate 1. Immunofluorescence microscopy of a cross-section of human skin exposed to saturated sulfur mustard vapor (1 min at 27 °C; Ct \approx 1040 mg.min.m⁻³; upper panel) or sulfur mustard (100 μ M, 30 min at 27 °C; middle panel) and of unexposed skin (lower panel), using monoclonal antibody 1H10, directed against sulfur mustard adducts to human keratin, in a 1/50 dilution. The photographs are composed from an image obtained for FITC fluorescence (mainly emanating from the stratum corneum; green) and from an image obtained for propidium iodide fluorescence representing DNA (red) in the same cross-section. (Reprinted with permission from G.P. van der Schans *et al.*, *Chem. Res. Toxicol.* **15**, 21-25, 2002. Copyright (2002) American Chemical Society.)

CHAPTER 1

Introduction

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1 THE CHEMICAL WEAPONS CONVENTION (CWC)

The Convention on the prohibition of the development, production, stockpiling, and use of chemical weapons and of their destruction (the Chemical Weapons Convention, CWC) was signed on January 13, 1993, and entered into force on April 29, 1997. The CWC includes 24 Articles, the Annex on Chemicals, the Annex on Implementation and Verification (so-called Verification Annex), and the Confidentiality Annex. The Verification Annex, which by the length occupies the majority of the CWC, is written in 11 parts. Article I lists the general obligations of the CWC as shown in Figure 1.

2 DEFINITIONS

The terms used in the CWC need to be explained, and for this reason, the terms used in the Articles

are defined in Article II, Definitions and Criteria. The terms used in the Verification Annex are defined in its first part. In general, a chemical weapon may be understood as munition filled with toxic chemical, but the definition of the CWC gives a larger perspective. Also, from the point of view of analytical chemistry, it is necessary to have an idea about what kind of chemicals we are aiming at. Also related to chemicals are a whole set of terms that need to be defined, that is, 'Chemical Weapons', 'Toxic Chemical', 'Precursor', and, in addition, 'key component'. The definitions are as follows.

'Chemical Weapons' means the following, together or separately: (a) toxic chemicals and their precursors, except where intended for purposes not prohibited under this Convention, as long as the types and quantities are consistent with such purposes; (b) munitions and devices, specifically designed to cause death or other harm through the toxic properties of those toxic chemicals specified in subparagraph (a), which would be released as

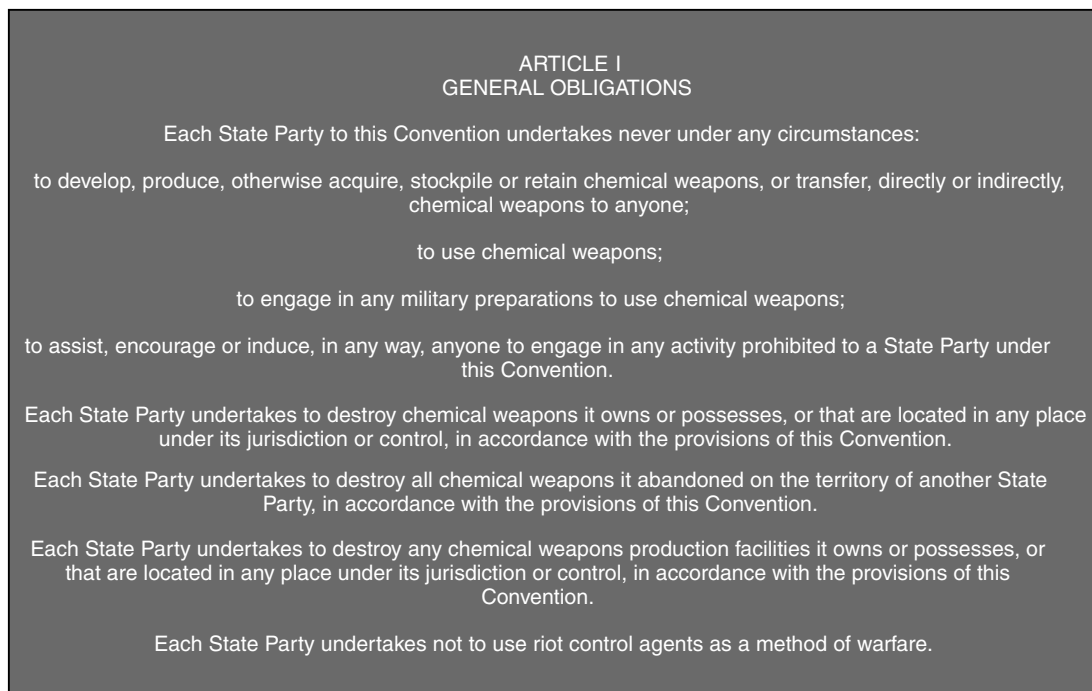


Figure 1. Article I of the Chemical Weapons Convention

a result of the employment of such munitions and devices; (c) any equipment specifically designed for use directly in connection with the employment of munitions and devices specified in (b).

‘Toxic Chemical’ means any chemical, which through its chemical action on life processes can cause death, temporary incapacitation, or permanent harm to humans or animals. This includes all such chemicals, regardless of their origin or of their method of production, and regardless of whether they are produced in facilities, in munitions, or elsewhere.

‘Precursor’ means any chemical reactant that takes part at any stage in the production, by whatever method, of a toxic chemical. This includes any key component of a binary or multicomponent chemical system.

‘Key Component of Binary or Multicomponent Chemical Systems’ (hereinafter referred to as ‘key component’) means any precursor, which plays the most important role in determining the toxic properties of the final product and reacts rapidly with other chemicals in the binary or multicomponent system.

3 SCHEDULES OF CHEMICALS

For the purpose of implementing the CWC, toxic chemicals and precursors, which have been identified for the application of verification measures, are listed in Schedules contained in the Annex on Chemicals (for the Schedules, see **Chapter 2**). Schedule 1 includes chemicals developed, produced, stockpiled, or used as a chemical weapon as defined above, and chemicals structurally close to them. Schedule 2 lists three toxic chemicals not included in Schedule 1 and the degradation products and precursors of these toxic chemicals as well as of those of Schedule 1. Schedule 3 lists four toxic chemicals and precursors not listed in the other Schedules. The Schedules contain mainly organic chemicals with different chemical and physical properties, being neutral chemicals, acids, bases, volatiles, and nonvolatiles, where phosphorus, fluorine, sulfur, chlorine, nitrogen, and oxygen occur frequently. Riot control agents are not included in the Schedules.

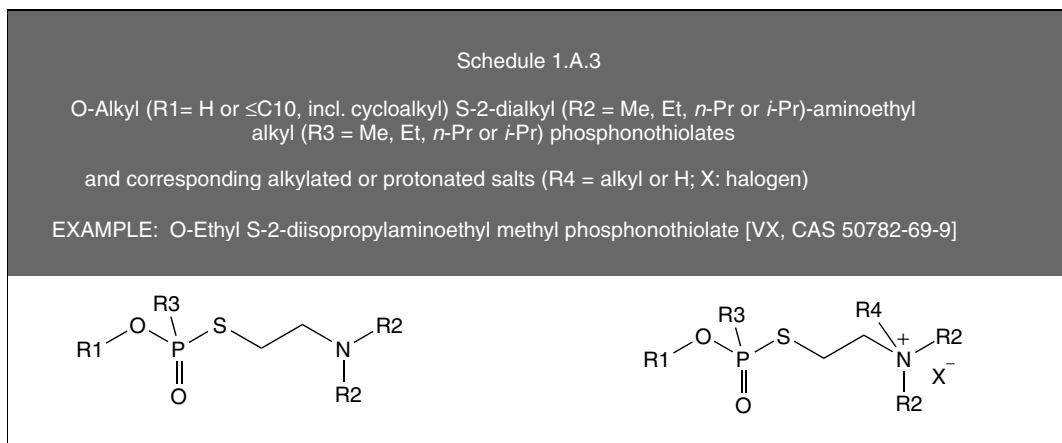


Figure 2. Definition and structures of Schedule 1.A.3 chemicals

The three Schedules contain altogether 57 list items from which 42 are individual chemicals and 15 are families of chemicals with a common structural backbone. Such families in the Schedules make the number of chemicals that are subject to verification very large. An idea of the number of chemicals in the Schedules may be obtained when considering, for example, the family of VX (Schedule 1.A.3), including its salts (Figure 2).

Another example is found in Schedule 2.B.4, which contains an even larger number of chemicals defined in the following way: ‘Chemicals, except for those listed in Schedule 1, containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms [exemption: Fonofos, CAS 944-22-9]’.

4 ORGANIZATION FOR THE PROHIBITION OF CHEMICAL WEAPONS (OPCW)

The States Parties to the CWC have established the Organization for the Prohibition of Chemical Weapons (OPCW; www.opcw.org) to achieve the object and purpose of the CWC, to ensure the implementation of its provisions, including those for international verification of compliance with it, and to provide a forum for consultation and cooperation among States Parties (SPs). All SPs to the CWC

are members of the OPCW. The Organization shall conduct its verification activities provided under the CWC in the least intrusive manner possible, consistent with the timely and efficient accomplishment of their objectives. It shall request only the information and data necessary to fulfill its responsibilities under the CWC. It shall take every precaution to protect the confidentiality of information on civil and military activities and facilities coming to its knowledge in the implementation of the CWC and, in particular, shall abide by the provisions set forth in the Confidentiality Annex. In undertaking its verification activities, the OPCW shall consider measures to make use of advances in science and technology.

5 VERIFICATION

The OPCW performs verification activities on a regular basis and can conduct challenge inspections. The purpose is to verify that the SPs fulfill their obligations under the CWC. Regular verification includes assessment of the declarations made by the SPs by conducting on-site inspections of declared sites.

The general rules for verification (Verification Annex, Part II, paragraphs 52–54) describe sample taking (sampling, sample collection) and analysis. By way of example, sampling and analysis shall be undertaken to check for the absence of undeclared scheduled chemicals during inspections under

regime of Schedule 2 chemicals and facilities related to such chemicals. Also, sampling and on-site analysis may be undertaken to check for the absence of undeclared scheduled chemicals during inspections under regime of Schedule 3 chemicals and facilities related to such chemicals. In case of unresolved ambiguities, samples may be analyzed in a designated off-site laboratory, subject to the inspected SPs agreement. (For the summary on the sampling and analysis in the CWC, *see* Annex 1 in **Chapter 2**.)

For the on-site analysis, the inspectors bring with them mobile instrumentation capable of performing the analysis in the least intrusive manner where the chemicals are revealed only according to the purpose of the inspection and the information on nonscheduled chemicals will remain confidential (for so-called blinded analysis, *see* **Chapter 4**).

For the off-site analysis, the designated laboratories are used. These laboratories have instrumental capability, preparedness, and analytical methods to analyze the samples taken by the inspectors or by the inspected SP representatives. The samples sent (after the agreement of the inspected SP) to the off-site laboratory are coded, and therefore the laboratory receiving the samples will not know their origin. The laboratories are capable of confirming the presence or absence of CWC-related chemicals and other chemicals, but must report only data relevant to the purpose of the analysis as defined by the OPCW. The laboratory's work on the OPCW samples is confidential, which is a normal practice when regarding the work with laboratory's other collaborators and commercial business partners. The work is reported only to the OPCW.

6 THIS BOOK

Chemical Weapons Convention Chemicals Analysis discusses sample collection, sample preparation and analysis, and concentrates on verification that takes place on site, analyses off site, and methods and procedures used. In the first part of the book is discussed the mobile laboratory of the OPCW and instrumentation and software used therein, as well as other on-site analysis equipment, procedures, and strategies. The OPCW gas chromatograph–mass spectrometer for on-site analysis is described and

an introduction to Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) software is given. Various monitoring methods of hazardous substances are viewed. A comprehensive review to 10 OPCW proficiency tests has been done. The topics related to the OPCW Central Analytical Database (OCAD) are discussed.

The second part of the book begins with a discussion of the analysis strategy employed in an OPCW-designated laboratory and continues with a discussion on sample preparation methods in an off-site laboratory and concludes with discussion on the various analytical techniques used for analysis of CWC-related chemicals in (designated) laboratories worldwide. The analytical techniques are gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), nuclear magnetic resonance (NMR) spectroscopy, gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR), and capillary electrophoresis (CE). The methods included in this part provide the best off-site performance for unambiguous identification of CWC-related chemicals. The success of these off-site analysis techniques has been unequivocally confirmed in the international proficiency tests. Other examples in the literature exist from excellent performance from 'real samples', for example, the detection of intact sarin by mass spectrometry from a painted metal fragment after four years of contamination.

In the third part, methods for retrospective detection of exposure to toxic scheduled chemicals using mass spectrometric and immunochemical analysis methods are discussed. The described methods are applied to human origin samples. These methods are essential when in cases of use, or allegations of use, previous presence or absence of toxic chemicals need to be confirmed. Identification of CWC-related chemicals provides key supporting evidence of noncompliance with the CWC.

ABBREVIATIONS AND ACRONYMS

AMDIS	Automated Mass Spectrometry Deconvolution and Identification System
CAS	Chemical Abstracts Service

CE	Capillary Electrophoresis	NMR	Nuclear Magnetic Resonance
CWC	Chemical Weapons Convention	OCAD	OPCW Central Analytical Database
GC	Gas Chromatography	OPCW	Organization for the Prohibition of Chemical Weapons
GC/FTIR	Gas Chromatography/Fourier Transform Infrared Spectroscopy	SPs	States Parties
GC/MS	Gas Chromatography/Mass Spectrometry	VX	<i>O</i> -Ethyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate
LC/MS	Liquid Chromatography/Mass Spectrometry		

CHAPTER 2

Sampling and Analysis in the Chemical Weapons Convention and the OPCW Mobile Laboratory

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1 INTRODUCTION

During the first session of the Preparatory Commission for the Organization for the Prohibition of Chemical Weapons (OPCW), which was held between 8 and 12 February 1993 in The Hague, Working Group B was established with the task of drafting procedures for ‘verification and technical cooperation and assistance’. Up to the entry

into force of the Chemical Weapons Convention (CWC) in April 1997, Working Group B developed procedures for the conduct of verification activities and specifications for equipment. An important part of the group’s deliberations was the issue of sampling and analysis (S&A). On the basis of the recommendations of Working Group B, the First Conference of States Parties, held in The Hague in May 1997, adopted a list of approved

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

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equipment for inspection purposes and procedures relating to proficiency testing for the designation of laboratories for off-site analysis, thus setting the initial conditions for sampling and analysis. On the basis of this, the Technical Secretariat (Secretariat) has developed its on-site and off-site S&A capability. The following article describes the role of on-site analysis in the verification process and the OPCW mobile laboratory to be used by inspectors to perform such on-site analysis. Particular emphasis is given to the description of software tools that were developed to protect confidential information (commercial or military) and which limit the analysis capability to CWC-related chemicals.

2 ROLE OF ON-SITE ANALYSIS IN THE VERIFICATION PROCESS

The CWC ⁽¹⁾ makes extensive reference to S&A in its 'Annex on Implementation and Verification (Verification Annex)'. First in part II, under General Rules of Verification, but also in later sections that describe verification activities for particular types of inspections or inspectable facilities as shown in Table 1. The full wording of the respective provisions is included in Annex 1.

S&A is one of several verification tools available to an inspection team (IT), and like any other, its purpose is to assist the IT in achieving the inspection mandate that was issued by the Director-General. From among all the tools available to the IT, S&A is

special in the sense that it provides factual evidence for the presence of scheduled chemicals through detection and identification and/or supports a conclusion of absence of scheduled chemicals through analysis results. Considering the role that was given to S&A in the CWC and the potential of S&A to provide factual evidence on the presence or absence of chemicals, it can be followed that the Secretariat of the OPCW must establish and maintain the capability to perform chemical analysis.

The CWC provides for three principal ways to undertake chemical analysis:

- (a) on-site analysis by the IT using approved inspection equipment [VA.II.53];
- (b) on-site analysis conducted by the inspected State Party ((ISP)) in the presence of the IT, using equipment available at the inspection site [VA.II.53]; and
- (c) off-site analysis at designated laboratories that have been certified by the Director-General for such analysis [VA.II.55].

The objective of S&A may depend on the type of inspection that is being conducted. However, the two standard inspection objectives of S&A are as follows:

- confirmation of the declaration (confirm identity of a declared chemical); and
- confirmation of absence of any undeclared scheduled chemicals, in particular, Schedule 1 chemicals.

While both objectives may pose particular challenges to S&A, the analysis for absence of any undeclared scheduled chemicals is significantly more demanding. The large number of chemicals that are theoretically possible to be synthesized based on the definitions in the Schedules of the CWC illustrate this in Table 2. A list of the Schedules of chemicals is included in Annex 2 ⁽²⁾.

Table 2 illustrates that the majority of *Schedule numbers* allow deriving only one or a few different possible chemicals. One *Schedule number* however, Schedule 2.B.4, describes millions of possibilities ('Chemicals, except for those listed in Schedule 1, containing a phosphorous atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but no further carbon atoms'); and more importantly, the theoretical possibilities for the nerve agents

Table 1. Provisions in the Verification Annex of the CWC for S&A. The full wording of the respective provisions is included in Annex 1. (Annotation: VA.II.52–58 reads as Verification Annex, part two, paragraph 52 to 58)

VA.II.52–58	General rules for S&A
VA.II.11(d),12	Inviolability of samples
VA.IV(A).49b,66,70	Storage, destruction facilities
VA.V.49(iii)	Production facilities
VA.VII.27	Schedule 2 facilities
VA.VIII.22	Schedule 3 facilities
VA.IX.19	Other chemical production facilities
VA.X.27c,36b,47,48	Challenge inspections
VA.XI.16–18	Investigations of alleged use
CA.16	Facility agreement: taking of samples and their analysis

Table 2. Estimated number of possible chemicals that can be derived from the definitions for scheduled chemicals contained in the Annex on Chemicals of the CWC not counting corresponding protonated or alkylated salts, where this is applicable

Schedule number	(Estimated) number of chemicals	Schedule number	(Estimated) number of chemicals	Schedule number	Number of chemicals
1.A.1	>20 000 ^a	2.A.1	1	3.A.1	1
1.A.2	>50 000 ^a	2.A.2	1	3.A.2	1
1.A.3	>200 000 ^a	2.A.3	1	3.A.3	1
1.A.4	9	2.B.4	Millions	3.A.4	1
1.A.5	3	2.B.5	20 ^b	3.B.5	1
1.A.6	3	2.B.6	100	3.B.6	1
1.A.7	1	2.B.7	1	3.B.7	1
1.A.8	1	2.B.8	1	3.B.8	1
1.B.9	4	2.B.9	1	3.B.9	1
1.B.10	>200 000 ^a	2.B.10	10	3.B.10	1
1.B.11	1	2.B.11	8	3.B.11	1
1.B.12	1	2.B.12	10	3.B.12	1
—	—	2.B.13	1	3.B.13	1
—	—	2.B.14	1	3.B.14	1
—	—	—	—	3.B.15	1
—	—	—	—	3.B.16	1
—	—	—	—	3.B.17	1

^aIncluding branched chains and cyclo alkane chains, not including bicyclo alkane chains and stereoisomers and not including corresponding protonated and alkylated salts

^bOnly including dichlorides and difluorides

in Schedule 1.A.1 to 1.A.3 exceed two hundred seventy thousand. Schedule 1.B.10 chemicals are precursors to Schedule 1.A.3 and respectively represent the same number of possibilities.

While only few of all these theoretically possible Schedule 1 chemicals have ever been stockpiled as chemical weapons, all of them are scheduled chemicals. In order for the Secretariat to be able to check for absence of any undeclared scheduled chemicals, the methods used for analysis should be capable of detecting and identifying as many of them as possible.

The CWC provides for different types of inspections and as stated above, the actual role of S&A in a particular inspection depends on the type of inspection being conducted. Table 3 lists types of inspections provided for in the CWC and the particular objective of S&A for each one.

The role of S&A at a Chemical Weapons Destruction Facility (CWDF) is different than in other types of inspections. At a CWDF, the main objective of S&A is to confirm the identity of a particular chemical that has been declared, that is, the declared chemical agent that is being destroyed. Further,

Table 3. Different types of inspections and the respective purpose of S&A

Type of Inspection	Main purpose of S&A
Inspection of Schedule 1 facility (other facility)	Confirm absence of nondeclared activity
Inspection of Schedule 2 facility	Confirm absence of nondeclared chemicals, in particular, Schedule 1 chemicals
Inspection of Schedule 3 facility	
Other chemical production facilities	Tagging of items for later S&A at CWDF
Chemical weapons storage facilities	Confirm identity of chemical agent being destroyed, confirm nondiversion of agent and end point of destruction of chemical agent
Chemical weapons destruction facilities (CWDF)	
Challenge inspections	Confirm absence of any nondeclared scheduled chemical(s)
Investigation of alleged use	Confirm absence of chemical weapons and riot control agents

S&A's objective is also to confirm that this chemical is not contained in the effluent streams above a certain concentration. This difference in the objective of S&A compared to that of confirming absence of nondeclared scheduled chemicals has influenced the approach taken by the Secretariat for the conduct of on-site analysis.

The next section explains how the two approaches, analysis of a declared chemical and analysis for undeclared scheduled chemicals, have influenced on-site analysis activities and how on-site laboratories are or may be used for such analysis.

It must be stated however, that the CWC would also allow for a different interpretation of the purpose of S&A at a CWDF. Paragraph 66 (c) of part IV (A) states that 'the specific type and quantity of chemical weapons being destroyed' should be part of systematic on-site verification measures. This would also allow analyzing for any undeclared scheduled chemicals being destroyed in addition to the declared chemical.

3 LABORATORIES FOR ON-SITE ANALYSIS

Sample collection will generally be conducted by the inspected State Party (during investigations of alleged use (IAU), the inspection team may collect the samples itself), however, the IT may collect the sample(s) if agreed in advance between the inspected State Party and the IT [VA.II.52]. The IT has the right to conduct sample analysis using its own approved equipment or it may witness analysis performed by the inspected State Party [VA.II.53]. This leads to *two different concepts* for on-site analysis as laid out below.

It is important to understand that OPCW inspectors must be able to demonstrate to all States Parties that all analysis results have been obtained on independent and verifiable bases, whilst remaining flexible to specific site conditions and requirements.

3.1 On-site Analysis Conducted by the Inspected State Party and Witnessed by the Inspection Team (IT)

If the analysis is conducted by the inspected State Party, OPCW inspectors are in the role of witnessing

the analysis performed by the site personnel. This has various implications because analytical technique, sample preparation and analysis procedures, and quality assurance and quality control measures may be different to the ones described in OPCW procedures.

From a practical point of view, it may be rather difficult to verify the proper operation of computer programs or the identity of chemicals used by site personnel, and, as a consequence, the correctness of analysis results. This, in particular, if the analysis is checking for undeclared scheduled chemicals and not aiming to confirm the presence of a declared scheduled chemical. In order for an analysis for absence of undeclared scheduled chemicals to be credible for verification purposes, it must be conducted in accordance with OPCW procedures, fulfilling OPCW QA/QC (quality assurance/quality control) criteria and using the OPCW Central Analytical Database (OCAD) as reference ⁽³⁾. [The OCAD contains peer-reviewed validated analytical data of Scheduled chemicals (mass spectra, gas chromatography retention indices, infrared spectra, nuclear magnetic resonance spectra) that has been approved for inclusion into the database by the policy making organs of the OPCW.]

Therefore, on-site analysis conducted by the inspected State Party based on its own procedures is used routinely only in CWDF. The main focus of analysis at CWDF is to confirm the identity of the agent being destroyed and its absence in the effluent streams as described above. *Agent identity* is confirmed by GC/MS (gas chromatograph/mass spectrometer) analysis of an agent sample; mass spectrum and retention index are compared to the OCAD. Alternatively, in place of retention index comparison, the retention time of the agent peak is compared to the retention time of an agent reference standard provided by the inspected State Party. *Absence of agent in effluent streams* is frequently proven by GC analysis using absence of a peak in the retention time window of the agent. The retention time window has been established by analyzing the standard described above. If the analysis requires sample preparation, such as solvent extraction, an aliquot of the sample matrix is spiked with the reference standard within the detection range of the method and analyzed in parallel for QA/QC purposes.

3.2 On-site Analysis Conducted by the IT

If the IT is conducting on-site analysis using its own equipment, the view taken has been that the team should be independent and carry the entire equipment necessary. This led to the design of the OPCW mobile laboratory, which is described in Section 4.

The preparation of the samples for analysis and the analyses itself will be performed by the IT using its own equipment approved for this purpose. Sample preparation and analysis is conducted in accordance with OPCW standard operating procedures (SOP) and work instructions (WI) applying OPCW QA/QC criteria.

Within this approach lays the possibility of various levels of assistance by the inspected State Party offering equipment, such as the use of inspected site laboratory facilities, fumed hoods, and the like. Such offers are laid down in facility agreements concluded between the inspected site and the OPCW, following an initial inspection. However, fundamental to this approach is that sample preparation and analysis are conducted by OPCW inspectors according to OPCW-approved procedures with equipment that meets OPCW QA/QC criteria. All activities may be witnessed by inspected State Party representatives.

The following section describes the design and capabilities of the OPCW mobile laboratory.

4 THE OPCW MOBILE LABORATORY

The OPCW mobile laboratory is designed for use in all types of inspections. It is able to function self-contained, if necessary in a tent powered by electricity generators. It contains sufficient equipment to allow the IT the collection and preparation of various types of sample matrices and the GC/MS analysis of their extracts. All items of equipment are packaged in *flight cases* in such a way that two persons can move each transport container by hand.

4.1 Equipment Requirements and Specifications

Any item of equipment that is carried by an OPCW IT must have been approved for this purpose. All

equipment that is currently used or allowed for use in inspections is contained on a *list of approved equipment* that was adopted by the Conference of States Parties at its first sessions in May 1997, in decision 71 (C-I/DEC.71)⁽⁴⁾. This list of approved equipment was the result of extensive negotiations held during the period of the preparatory commission of the OPCW in the expert groups of Working Group B. For each item of equipment ‘general and specific operational requirements, common evaluation criteria and technical specifications’ were defined in Annexes to C-I/DEC.71. In C-I/DEC.71, individual items are listed together according to their intended purpose in particular equipment kits. An excerpt of C-I/DEC.71, the GC/MS sample preparation kit, is presented as an example in Annex 3.

Following is a description of the purpose and design of the OPCW mobile laboratory and its respective equipment kits. While the list of approved equipment in C-I/DEC.71 contains other items for S&A (i.e. FTIR, Alleged Use Sample Collection Kit), the items described in this article are the ones that have been actually procured and are actively used by OPCW ITs at the time of this publication. A more detailed description of S&A equipment kits can be found in **Chapter 3**.

4.2 Sample Collection Kit

The sample collection kit has been designed to allow the IT to collect bulk solid, soil, water, liquid and wipe samples. It contains enough items to collect eight samples of each matrix. All items intended to come into contact with the sample are packed individually for one time use. The items are arranged in transport boxes (flight cases) in individual drawers (Picture 1). Empty ‘pelli cases’ are included to allow the inspectors to assemble and carry forward specific items to the sampling location suitable for the particular sampling activity. A reduced version of the sample collection kit packaged in a briefcase is available for inspections where this will suffice.

The Secretariat developed sample collection procedures in accordance with its quality system that describe the collection of different types of samples (see Table 4).



Picture 1. Some items of sample collection kit

Table 4. Secretariat quality documents describing the collection of samples during on-site inspection. The documents listed in the table are part of the Secretariat's quality management system and undergo regular revision. Thus, their names and codes may change in future

Work instruction for:	Secretariat reference
Collecting and splitting of toxic samples under hazardous conditions on-site	QDOC/LAB/WI/SC1
Collection and splitting of samples under non-hazardous conditions	QDOC/LAB/WI/SC2

4.3 Sample Preparation Kit

The sample preparation kit (Picture 2) holds all equipment necessary to prepare the samples for GC/MS analysis on-site in four transport boxes (flight cases). It contains everything from the pH paper to the laboratory coat including the chemicals to conduct the sample preparation procedures. A reduced version of the sample preparation kit packaged in two pelli cases is available for inspections where this will suffice.

The objective of all sample preparation procedures is to extract the analytes of interest from the different sample matrices and then to bring them



Picture 2. Sample preparation kit

into a form that they can pass through a GC column, using derivatization if required. One of the key items of the kit and next to the fume hood the most bulky one is the centrifugal evaporator that allows a gentle evaporation of water in order to isolate polar analytes that remain in the water fraction after solvent extraction. The Secretariat drafted generic sample preparation procedures as shown in Table 5. These procedures use liquid/liquid and solid/liquid extraction and are with slight modifications based on the VERIFIN Blue Books ⁽⁵⁾. A detailed description of the sample preparation kit and methods can be found in **Chapter 3**.

4.4 Sample Transport Kits

The list of approved equipment contains two sample transport containers designed for the transport of samples for off-site analysis. The two containers are labelled as 'large- and small sample transport kits' (Pictures 3 and 4) and both are designed to fulfill the requirements for air transport [IATA (International Air Transport Association) provision A106] ⁽⁶⁾ and international standards for road, railway, and sea transport. The large container has been designed and tested by the United States and the small container by the United Kingdom. Therefore, the two containers are frequently referred to as US and UK containers.

Table 5. Secretariat quality documents describing the preparation of samples during on site inspection for GC/MS analysis, the destruction of sample material and the chain of custody documentation. The documents listed in the table are part of the Secretariat's quality management system and undergo regular revision. Thus, their names and codes may change in future

Work instruction for:	Reference
Preparation of samples on-site for GC/MS analysis	QDOC/LAB/WI/SP2
The chain of custody and documentation for OPCW samples on site	QDOC/LAB/WI/OSA3
The destruction of sample material	QDOC/LAB/WI/SP016

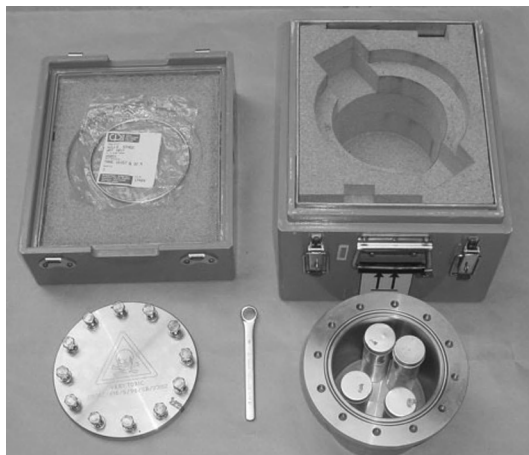


Picture 3. Large sample transport kit

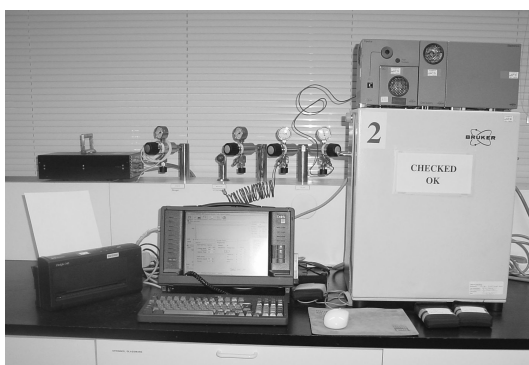
The small container is approved for the transport of 10 g of pure material or 400 g of diluted material and the large container is approved for 350 ml of any material.

4.5 GC/MS

The OPCW mobile laboratory includes a portable GC/MS. The system shown in Picture 5 including printer and helium connection kit is packed in five transport boxes. Because of the modular design of the instrument, it is shipped with two GC-ovens and two GC-injectors in order to allow the



Picture 4. Small sample transport kit



Picture 5. Bench top GC/MS instrument

inspector on-site to change the inlet and separation part should problems occur. At the time of writing, the Secretariat was in the process of replacing the GC/MS shown in Picture 5 with a standard bench top instrument adapted for OPCW purposes. Before any GC/MS is packed and dispatched from the Secretariat it is tested at the OPCW Laboratory in accordance with the Secretariat's quality system. A detailed description of the testing procedure and the instrument QA/QC criteria can be found in **Chapter 4**. For each GC/MS to be dispatched a certificate of the office of the internal oversight

(OIO) of the Secretariat is issued to confirm that the system meets all relevant performance criteria. Both organizational units, the OPCW Laboratory and the OIO, are accredited by the Dutch Accreditation Council (RvA) for this activity.

Two types of portable analytical equipment were approved by the Conference of State Parties for on-site analysis, that is, GC/MS and Fourier transform infrared (FTIR). Initially, it was anticipated that FTIR might be used in storage, destruction and Schedule 1 facilities for screening purposes to confirm the presence of declared chemicals. While FTIR analysis is suitable to identify pure chemicals or certain chemicals in mixtures at varying detection limits, FTIR cannot be used to analyze for absence of undeclared scheduled chemicals, for which the IT would have to carry in addition to the FTIR a GC/MS. For GC/MS analysis samples are analyzed at very low concentration contrary to FTIR analysis, which minimizes the risk of contamination in the on-site laboratory and reduces the risk of exposure of OPCW inspectors and on-site personnel. Considering the limitations of FTIR and the fact that GC/MS can cover the field of application of the FTIR for OPCW purposes, a decision was taken in 2000 to focus on the use of GC/MS in the OPCW mobile laboratory subject to future developments.

The Secretariat developed a set of quality documents for the preparation and handling of the GC/MS system (see Table 6).

Table 6. Secretariat quality documents describing the preparation and handling of the GC/MS system. The documents listed in the table are part of the Secretariat's quality management system and undergo regular revision. Thus, their names and codes may change in future

Work instruction for:	Reference
Bruker EM 640S portable GC/MS on-site analysis	QDOC/LAB/WI/GCMS001
Bruker EM 640S GC/MS testing and preparation of instruments for on-site analysis	QDOC/LAB/WI/GCMS002
Bruker EM 640S portable GC/MS packing procedures	QDOC/LAB/WI/GCMS003
Bruker EM 640S portable GC/MS installation and handling of software	QDOC/LAB/WI/GCMS004

5 BLINDING OF THE GC/MS INSTRUMENT

Confidentiality concerns, in particular, in relation to inspections of industry facilities and in relation to activities not prohibited by the Convention have led to several measures aimed at protecting sensitive information. In order to allow the inspected State Party to retain all data produced by the GC/MS, the equipment is operated from a removable hard disk, which may be retained on site at the end of the inspection. Another measure has been the design of dual mode software for the GC/MS by modifying the operating software of the instrument to offer operation in so-called open or blinded mode as explained in the following.

Open mode provides standard functionality of the GC/MS; blinded mode limits the information that is revealed by the instrument to the operator. If operated in blinded mode, the instrument will only display or reveal information on those chemicals contained in the sample that are relevant to assess presence or absence of scheduled chemicals. In order to achieve this the data postprocessing software can only search a specific mass spectral library that is an extract of the OCAD. This OCAD only contains chemicals that have been approved for inclusion into the database by the policy making organs of the OPCW. For a full description of the OCAD, please refer to **Chapter 7**. Two independent software modules were developed, which used independently or together limit the information that is displayed by the instrument. One is the *blinding feature* of the instrument operating system and the other the *negotiation module* of the data postprocessing software AMDIS (Automated Mass Spectral Deconvolution and Identification System developed by the United States Department of Commerce, National Institute of Standards and Technology (NIST)), which provides for different so-called *security levels*.

During the set-up process of the GC/MS instrument that is shown in Picture 5, the negotiation module is used to install the *on-site target library* and to select the *security level filter* of AMDIS (this approach may be modified for the new GC/MS system). The *security level filters* of AMDIS progressively restrict the accessibility to and the content of the data that AMDIS provides after post-processing of the spectral data. In order to protect

the selections made in the negotiation module, the IT and the inspected State Party enter a password. Any changes require both passwords to be entered. After installation of the library and selection of the security level filters, the decision must be made whether the instrument is to be operated in blinded mode or in open mode; in the first case, the blinding feature is activated; in the latter it is not.

Figure 1 shows schematically the relationship between *on-site target library*, *blinding*, and *AMDIS security level filter*.

The center of Figure 1 (not shaded) shows the GC/MS instrument 640S GC/MS allowing to *monitor method parameters* and producing *on-line data*; at the end of the analysis, *triggering* data processing with *AMDIS on-site version* and allowing for *postrun data* processing.

On the left side, the window labelled *available in open mode only*, displays features available with a standard GC/MS. This includes a *file manager* to handle files, a *word processor* to edit files, *display of online data* during analysis (total

ion chromatogram (TIC) and mass spectrum) and after processing of *postrun data* using one of the two installed data analysis software: *Bruker DA* or *AMDIS open version*.

On the right side the window labelled *AMDIS on-site version* displays 5 security level filters of AMDIS gradually limiting the information displayed to the operator as described in Table 7.

- A *source library* on 3.5 in. floppy disk or CD-ROM is prepared at the OPCW Laboratory from the OCAD and issued with a certificate of authenticity from the OIO together with the GC/MS instrument to the IT. This source library contains mass spectra and retention indices data from the OCAD.
- In the *negotiation module*, the chemicals from this *source library* that should be copied into the *on-site target library* can be selected on the basis of a chemical name or a schedule number; by default, the content of the *on-site target library* includes all chemicals of the *source library*. In order to install the mass

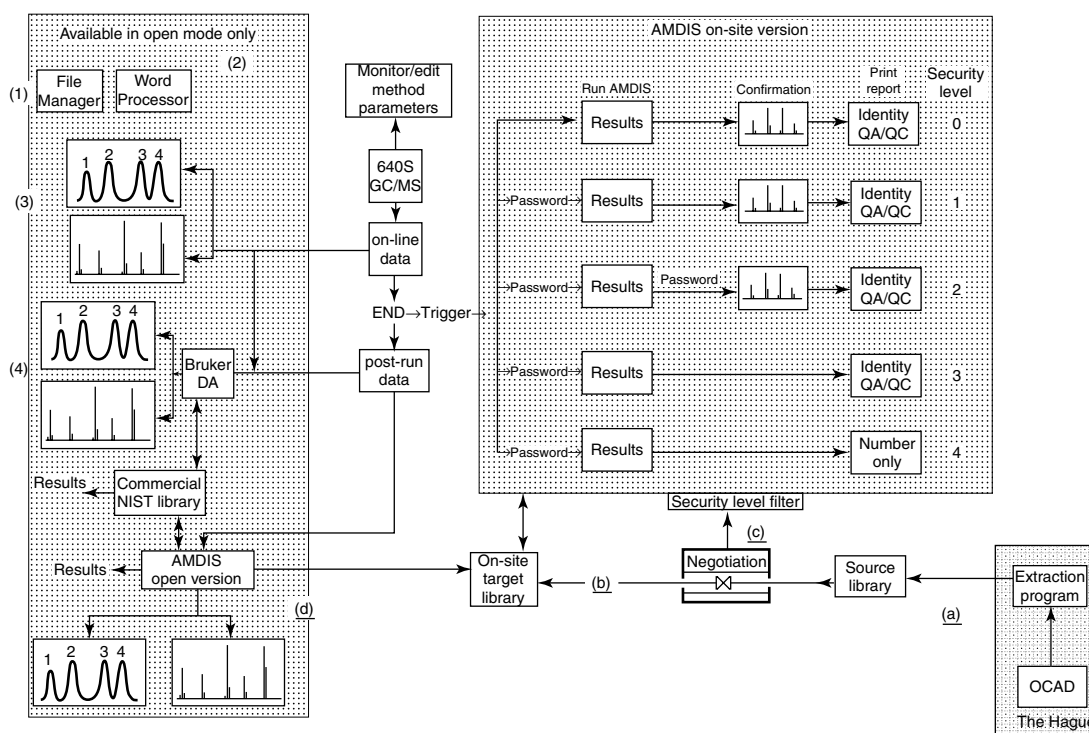


Figure 1. Relationship between *on-site target library*, *blinding*, and *AMDIS security level filter*

Table 7. The effects of the different AMDIS security level filters

Security level 0	<p><i>AMDIS on-site version</i> data analysis software is triggered automatically after each GC/MS run. The output includes names, net match factor, retention times and retention indices, and a set of quality assurance parameters, i.e. peak width, peak tailing, signal to noise ratio of each identified chemical and the internal standard. In addition, general gas chromatography QA/QC parameters such as background, solvent tailing and bleeding are reported.</p> <p>The software allows the opening of the <i>AMDIS confirmation window</i>, which displays the TIC of identified peaks, mass spectra at peak maximum and extracted spectrum after deconvolution; and the corresponding mass spectrum of a library hit in the <i>on-site target library</i>.</p>
Security level 1	Includes the same features as security level 0. Further, entering of both passwords is requested to start <i>AMDIS on-site version</i> data analysis software following a GC/MS analysis run. Because of this, data analysis can only be performed in the presence of both IT and ISP.
Security level 2	In addition to security level 1, the opening of the <i>AMDIS confirmation window</i> , which displays the TIC of identified peaks and their mass spectra requires the passwords of the ISP and IT. Because of this, again, data analysis can only be performed in the presence of both IT and ISP.
Security level 3	In this security level, the <i>AMDIS confirmation window</i> is no longer available. As a result, the IT can no longer compare mass spectra of an analyte identified by AMDIS to the respective library spectra.
Security level 4	The restrictions of security level 3 apply. In addition, AMDIS only reports how many chemicals it identified. No further information is provided such as chemical names, retention time/indices and QA/QC parameters. An internal standard, which is injected with each analysis should always be identified by AMDIS. However, because AMDIS will only report i.e. 'one chemical identified' it remains unknown if the internal standard or any other chemical was in fact detected; thus, this security level produces results of very limited value.

Table 8. Effects of operating instrument in 'blinded' mode as indicated in Figure 1

Effects of blinding	Effects on analysis
(1) File manager deleted	No copying of files possible
(2) Word processor deleted	No editing of files
(3) No display of real-time data	No display of TIC or mass spectra on PC screen during data acquisition
(4) Bruker mass spectral data analysis software and AMDIS open version deleted	<p>The instrument is issued by the OPCW Laboratory with three data postprocessing software tools:</p> <ul style="list-style-type: none"> • Bruker mass spectral data analysis software from the instrument provider; • <i>AMDIS open version</i>, a full version of AMDIS mass spectral data analysis software; and • <i>AMDIS on-site version</i>, mass spectral data analysis software, which is triggered at the end of each analysis run. This reduced on-site version of AMDIS only displays TICs and mass spectra of analytes that match a chemical in the on-site target library. <p>As a result only peak(s) and mass spectra of analytes identified by AMDIS in the on-site target library are displayed</p>
(5) No search of libraries other than on-site target library.	The library search function is altered. AMDIS on-site version can only search in the on-site target library.

spectra library onto the GC/MS computer hard disk, the passwords entered in the negotiation module are required (inspected State Party and IT). As a result, it is not possible to change the content of the *on-site target library* without both parties entering their respective passwords.

- (c) The *security level filter* of *AMDIS on-site version* is selected with the effects as indicated in Table 7. The *security level filters* may be changed at any time by entering the required passwords. Data of a previous analysis may be reanalyzed at a different security level.
- (d) If the software switch for *blinded mode* is selected, the effects will be as indicated in Table 8. Contrary to the *AMDIS security level filters*, blinding is irreversible on a particular PC hard disk. Returning the instrument to open mode requires formatting of the hard disk and reinstallation of all operating software. In order to facilitate the return of the system into open mode during an inspection, the instrument is dispatched with two removable hard disks. This allows the IT to replace a blinded mode hard disk and to reanalyze the samples in open mode if necessary.

At the time of writing, a new blinded mode software was being developed. This new version is based on different GC/MS operating software and will include additional security features such as unique installation numbers and check sum. Also, the need of *AMDIS security level filters* is being reviewed.

6 LIMITATIONS OF THE OPCW MOBILE LABORATORY

The OPCW mobile laboratory equipment has been selected to allow operation of a GC/MS laboratory on site, aiming at a performance similar to the one of a stationary laboratory using a standard bench top instrument. However, restrictions in the selection and operation of the equipment (i.e. protection of confidential information) and issues related to logistics/transport of the laboratory equipment cause certain limitations, which are described in the paragraphs below.

The capabilities of the mobile laboratory depend to a large extent on the equipment and procedures that are approved for the use on site. Limitations are described best by discussing them separately for sample collection, sample preparation, GC/MS equipment, and analysis procedures.

6.1 Sample Collection Equipment

The equipment approved by the first Conference of States Parties in C-I/DEC.71 for collecting samples for on-site analysis is sufficient and adequate to collect samples of various types of matrices, environmental, and bulk. The sample collection kit as it is designed now allows for the collection of a limited number of samples of each type, about eight, but could be extended if there was a need. The key limitation in relation to sample collection at this point in time is that the kit does not contain equipment for the collection of air samples and that there are no respective procedures in place. A more general limitation is the very detailed specifications in the list of approved equipment given for each equipment item, rendering any change or development impossible without approval by the policy-making organs.

6.2 Sample Preparation Equipment

This has particular limiting effects for sample preparation because of the detailed specifications given for equipment and chemicals approved for the sample preparation kit. The items of the sample preparation kit and not their purpose have been specified in detail. It turned out that some items, originally approved on the list, where not available on the market, except if custom produced for the OPCW. Because of these detailed specifications, developments in the area of sample preparations cannot easily be applied to on-site analysis. As a result, any change to sample preparation procedures that require an additional item of equipment, or more so an additional chemical, such as a different solvent (i.e. for sample clean up), cannot be implemented until the list of approved equipment is amended.

Current sample preparation procedures for water samples use low temperature vacuum evaporation of water with a centrifugal evaporator followed by derivatization. They are time consuming and

require bulky equipment. Any sample preparation procedure that would allow obtaining/derivatization of analytes without slow elimination of water would greatly speed up the sample preparation process and reduce the amount of equipment that has to be carried.

The list of chemicals approved to be brought by the IT and to be used in the OPCW mobile laboratory on site contains no scheduled chemicals in order to prevent any contamination of on-site samples. Therefore, the IT cannot compare analytes detected by GC/MS analysis to reference standards or synthesize these standards on site.

6.3 GC/MS instrument

The specifications approved for the GC/MS instrument allow the selection of an instrument with performance criteria of a standard GC/MS bench top system. The requirements specified in relation to blinding and transportability are substantial and require significant adaptations to any system currently available on the market. However, they do not limit analysis performance in terms of sensitivity or selectivity. The current system specifications do not include chemical ionization and limit analysis to electron impact spectra.

The system is dispatched with a commercial spectral database and a copy of the OCAD, which are both available if analysis is conducted in open mode. If the instrument is operated in blinded mode and AMDIS security level filters are applied, the limitations as outlined in Section 5 above apply.

The content of the OCAD is currently limited to scheduled chemicals, their degradation products and some derivatives, and depends on the data contributed to the OPCW by Member States. The current version of the OCAD (June 2004) is sufficient for on-site analysis; it contains the mass spectra of about 2000 chemicals. A detailed description of the OCAD can be found in **Chapter 7**.

7 THE OPCW S&A NETWORK

While the OPCW mobile laboratory is an important tool in the verification process, it is only one element of the OPCW S&A network. In Section 1, it was

explained that the CWC provides for three principal ways to undertake chemical analysis, one of them being off-site analysis at designated laboratories. The CWC provides for off-site analysis for all types of inspections but off-site analysis is subject to agreement of the inspected State Party in the case of Schedule 3 inspections and inspections of other chemical production facilities (OCPF). In the event of off-site analysis, the Director-General must select a minimum of two laboratories from a list of laboratories that have been designated by him.

7.1 OPCW Proficiency Testing

The OPCW Secretariat conducts official OPCW proficiency tests that lead to designation by the Director-General; under normal circumstances, two tests per year. Until July 2004, 15 such tests have been conducted. All Member States are invited to nominate laboratories to participate in these proficiency tests. For laboratories to become designated, they must participate successfully three times consecutively and they must obtain an accreditation for the analysis of chemical weapons-related chemicals by their national accreditation bodies. It is the aim of the OPCW to have designated laboratories from a geographical distribution as wide as possible. Currently, there are 18 laboratories designated in four regional groups. A comprehensive description of the OPCW proficiency testing can be found in **Chapter 6**.

7.2 The Role of the OPCW Laboratory in Rijswijk during Off-site Analysis

In the event of off-site analysis, authentic samples will pass from the inspection site through the OPCW Laboratory to designated laboratories. At the OPCW Laboratory, the samples will be unpacked from their transport container(s) to confirm identity by checking seal numbers and sample weight. Before the samples are repackaged and dispatched to designated laboratories, preanalyzed spiked control samples and matrix blanks from similar matrix (water, organic solvent, soil) are prepared at the OPCW laboratory for distribution together with the authentic samples. A designated laboratory receives for each sample, three nonindicated vials containing sample, spiked control, and blank. During the entire

process, the authentic samples remain sealed until their arrival at a designated laboratory. Off-site analysis results are assessed by the OPCW laboratory for correct analysis of control samples and blanks, for conforming to relevant reporting criteria and for consistency of results between designated laboratories.

ABBREVIATIONS AND ACRONYMS

AMDIS	Automated Mass Spectral Deconvolution and Identification System
BSTFA	Bis-(trimethylsilyl)trifluoroacetamide
CWC	Chemical Weapons Convention
CWDF	Chemical Weapons Destruction Facility
DMT	Dimercaptotoluene
FTIR	Fourier Transform Infrared
GC/MS	Gas Chromatograph/Mass Spectrometer
IATA	International Air Transport Association
IAU	Investigations of Alleged Use
ISP	Inspected State Party
IT	Inspection Team
NIST	National Institute of Standards and Technology
OCAD	OPCW Central Analytical Database
OCPF	Other Chemical Production Facilities
OIO	Office of the Internal Oversight
PFIB	1,1,3,3,3-Pentafluoro-2-trifluoromethyl-1-propene
QA/QC	Quality Assurance/Quality Control
QL	O-Ethyl O-2-diisopropylaminoethyl methylphosphonite
SOP	Standard Operating Procedures
THF	Tetrahydrofuran
TIC	Total Ion Chromatogram
VX	O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate
WI	Work Instructions

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ANNEX 1

Provisions for sampling and analysis in the Verification Annex of the CWC

PART II: General Rules of Verification

- 11. To exercise their functions effectively, inspectors and inspection assistants shall be accorded privileges and immunities as set forth in subparagraphs (a) to (i). . .
 - (d) Samples and approved equipment carried by members of the inspection team shall be inviolable subject to provisions contained in this Convention and exempt from all customs duties. Hazardous samples shall be transported in accordance with relevant regulations.
- 12. When transiting the territory of non-inspected States Parties, the members of the inspection team shall be accorded the privileges and immunities enjoyed by diplomatic agents pursuant to Article 40, paragraph 1, of the Vienna Convention on Diplomatic Relations. Papers and correspondence, including records, and samples and approved equipment, carried by them, shall be accorded the privileges and immunities set forth in paragraph 11 (c) and (d).
- 53. Where possible, the analysis of samples shall be performed on-site. The inspection team shall have the right to perform on-site analysis of samples using approved equipment brought by it. At the request of the inspection team, the inspected State Party shall, in accordance with agreed procedures, provide assistance for the analysis of samples on-site. Alternatively, the inspection team may request that appropriate analysis on-site be performed in its presence.
- 54. The inspected State Party has the right to retain portions of all samples taken or take duplicate samples and be present when samples are analysed on-site.
- 55. The inspection team shall, if it deems it necessary, transfer samples for analysis off-site at laboratories designated by the Organization.
- 56. The Director-General shall have the primary responsibility for the security, integrity and preservation of samples and for ensuring that the confidentiality of samples transferred for analysis off-site is protected. The Director-General shall do so in accordance with procedures, to be considered and approved by the Conference pursuant to Article VIII, paragraph

Collection, handling and analysis of samples

- 52. Representatives of the inspected State Party or of the inspected facility shall take samples at

21(i), for inclusion in the inspection manual. He shall:

- (a) Establish a stringent regime governing the collection, handling, transport and analysis of samples;
- (b) Certify the laboratories designated to perform different types of analysis;
- (c) Oversee the standardization of equipment and procedures at these designated laboratories, mobile analytical equipment and procedures, and monitor quality control and overall standards in relation to the certification of these laboratories, mobile equipment and procedures; and
- (d) Select from among the designated laboratories those which shall perform analytical or other functions in relation to specific investigations.

57. When off-site analysis is to be performed, samples shall be analysed in at least two designated laboratories. The Technical Secretariat shall ensure the expeditious processing of the analysis. The samples shall be accounted for by the Technical Secretariat and any unused samples or portions thereof shall be returned to the Technical Secretariat.

58. The Technical Secretariat shall compile the results of the laboratory analysis of samples relevant to compliance with this Convention and include them in the final inspection report. The Technical Secretariat shall include in the report detailed information concerning the equipment and methodology employed by the designated laboratories.

PART IV (a): Destruction of Chemical Weapons and its Verification Pursuant to Article IV

Inspections and visits

49. Inspectors shall, in accordance with facility agreements:

- (b) Have the right, during the first and any subsequent inspection of each chemical weapons storage facility, to designate munitions, devices, and containers from which samples are to be taken,

and to affix to such munitions, devices, and containers a unique tag that will indicate an attempt to remove or alter the tag. A sample shall be taken from a tagged item at a chemical weapons storage facility or a chemical weapons destruction facility as soon as it is practically possible in accordance with the corresponding destruction programmes, and, in any case, not later than by the end of the destruction operations.

Systematic on-site verification measures at chemical weapons destruction facilities

67. Inspectors shall have the right to tag, for sampling, munitions, devices, or containers located in the temporary holding areas at the chemical weapons destruction facilities.

70. Inspectors shall, in accordance with facility agreements: . . .

- (b) Monitor the systematic on-site analysis of samples during the destruction process; and
- (c) Receive, if necessary, samples taken at their request from any devices, bulk containers and other containers at the destruction facility or the storage facility there at.

PART V: Destruction of Chemical Weapons Production Facilities and its Verification Pursuant to Article V

Systematic verification of chemical weapon production facilities and cessation of their activities

49. The detailed facility agreement for each chemical weapons production facility shall specify:

- (a) Detailed on-site inspection procedures, which may include:
- (iii) Obtaining and analyzing samples;

PART VII: Activities not Prohibited Under this Convention in Accordance with Article VI

**Regime for Schedule 2
Chemicals and Facilities Related
to such Chemicals**

Inspection procedures

27. Sampling and analysis shall be undertaken to check for the absence of undeclared scheduled chemicals.

PART VIII: Activities not Prohibited Under this Convention in Accordance with Article VI

**Regime for Schedule 3
Chemicals and Facilities
Related to such Chemicals**

Inspection procedures

22. Sampling and on-site analysis may be undertaken to check for the absence of undeclared scheduled chemicals. In case of unresolved ambiguities, samples may be analyzed in a designated off-site laboratory, subject to the inspected State Party's agreement.

PART IX: Activities not Prohibited Under this Convention in Accordance with Article VI

**Regime for other Chemical
Production Facilities**

Inspection procedures

19. Sampling and on-site analysis may be undertaken to check for the absence of undeclared scheduled chemicals. In cases of unresolved ambiguities, samples may be analyzed in a

designated off-site laboratory, subject to the inspected State Party's agreement.

PART X: Challenge Inspections Pursuant to Article IX

Securing the site, exit monitoring

27. Additional procedures for exit monitoring activities as agreed upon by the inspection team and the inspected State Party may include, inter alia: ...

(c) Sample analysis.

Perimeter Activities

36. In conducting the perimeter activities, the inspection team shall have the right to: ...

(b) Take wipes, air, soil or effluent samples;

Managed Access

47. The inspected State Party shall designate the perimeter entry/exit points to be used for access. The inspection team and the inspected State Party shall negotiate: the extent of access to any particular place or places within the final and requested perimeters as provided in paragraph 48; the particular inspection activities, including sampling, to be conducted by the inspection team; the performance of particular activities by the inspected State Party; and the provision of particular information by the inspected State Party.
48. In conformity with the relevant provisions in the Confidentiality Annex the inspected State Party shall have the right to take measures to protect sensitive installations and prevent disclosure of confidential information and data not related to chemical weapons. Such measures may include, inter alia: ...

(e) Restriction of sample analysis to presence or absence of chemicals listed in Schedules 1, 2 and 3 or appropriate degradation products;

PART XI: Investigations in Cases of Alleged use of Chemical Weapons

Sampling

16. The inspection team shall have the right to collect samples of types, and in quantities it considers necessary. If the inspection team deems it necessary, and if so requested by it, the inspected State Party shall assist in the collection of samples under the supervision of inspectors or inspection assistants. The inspected State Party shall also permit and cooperate in the collection of appropriate control samples from areas neighboring the site of the alleged use and from other areas as requested by the inspection team.
17. Samples of importance in the investigation of alleged use include toxic chemicals, munitions and devices, remnants of munitions and devices, environmental samples (air, soil, vegetation, water, snow, etc.) and biomedical samples from human or animal sources (blood, urine, excreta, tissue etc.).

18. If duplicate samples cannot be taken and the analysis is performed at off-site laboratories, any remaining sample shall, if so requested, be returned to the inspected State Party after the completion of the analysis.

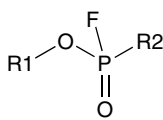
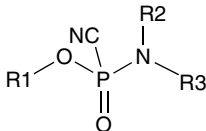
Confidentiality Annex

C. Measures to Protect Sensitive Installations and Prevent Disclosure of Confidential Data in the Course of On-site Verification Activities

16. In the elaboration of arrangements and facility agreements, due regard shall be paid to the requirement of protecting confidential information. Agreements on inspection procedures for individual facilities shall also include specific and detailed arrangements with regard to the determination of those areas of the facility to which inspectors are granted access, the storage of confidential information on-site, the scope of the inspection effort in agreed areas, the taking of samples and their analysis, the access to records and the use of instruments and continuous monitoring equipment.

ANNEX 2: SCHEDULES 1-3

Adapted from ref. (2), M. Mesilaakso, and M. Rautio, Verification of Chemicals related to the Chemical Weapons Convention, **Table 1**, Encyclopedia of Analytical Chemistry, Wiley, 2000

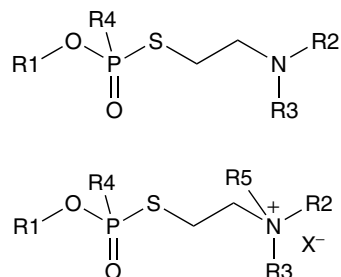
Schedule 1	
A. Toxic chemicals (CAS reg. No.)	Structure
(1) <i>O</i> -Alkyl ($R_1: \leq C_{10}$, incl. cycloalkyl) alkyl (R_2 : Me, Et, <i>n</i> -Pr or <i>i</i> -Pr)-phosphonofluoridates	
e.g. Sarin: <i>O</i> -Isopropyl methylphosphonofluoridate (107-44-8); Soman: <i>O</i> -Pinacolyl methylphosphonofluoridate (96-64-0)	
(2) <i>O</i> -Alkyl ($R_1: \leq C_{10}$, incl. cycloalkyl) <i>N,N</i> -dialkyl ($R_{2,3}$: Me, Et, <i>n</i> -Pr or <i>i</i> -Pr) phosphoramidocyanidates	
e.g. Tabun: <i>O</i> -Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate (77-81-6)	

(continued overleaf)

(continued)

Schedule 1

- (3) *O*-Alkyl (R_1 : H or $\leq C_{10}$, incl. cycloalkyl)
S-2-dialkyl ($R_{2,3}$: Me, Et,
n-Pr or *i*-Pr)-aminoethyl alkyl (R_4 : Me, Et,
n-Pr or *i*-Pr) phosphonothiolates and
 corresponding alkylated or protonated salts
 (R_5 : alkyl or H; X : anion)
 e.g. VX: *O*-Ethyl
S-2-diisopropylaminoethyl methyl
 phosphonothiolate (50782-69-9)



- (4) Sulfur mustards:

2-Chloroethylchloromethylsulfide
 (2625-76-5)

Mustard gas: Bis(2-chloroethyl)sulfide
 (505-60-2)

Bis(2-chloroethylthio)methane
 (63869-13-6)

Sesquimustard:
 1,2-Bis(2-chloroethylthio)ethane
 (3563-36-8)

1,3-Bis(2-chloroethylthio)-*n*-propane
 (63905-10-2)

1,4-Bis(2-chloroethylthio)-*n*-butane
 (142868-93-7)

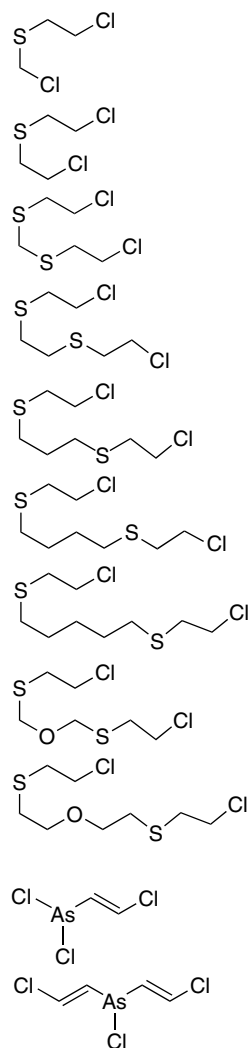
1,5-Bis(2-chloroethylthio)-*n*-pentane
 (142868-94-8)

Bis(2-chloroethylthiomethyl)ether
 (63918-90-1)

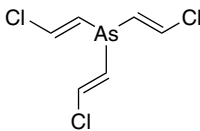
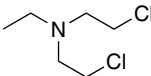
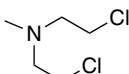
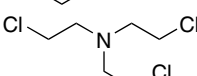
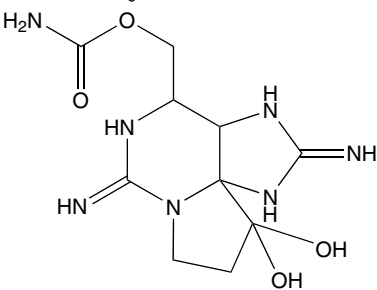
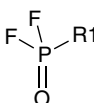
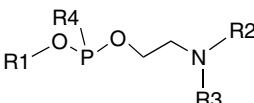
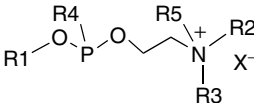
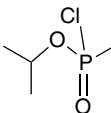
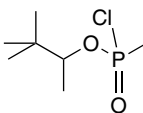
O-Mustard:
 Bis(2-chloroethylthioethyl)ether
 (63918-89-8)

- (5) Lewisites:
Lewisite 1: 2-Chlorovinylchloroarsine
 (541-25-3)

Lewisite 2: Bis(2-chlorovinyl)chloroarsine
 (40334-69-8)



Schedule 1

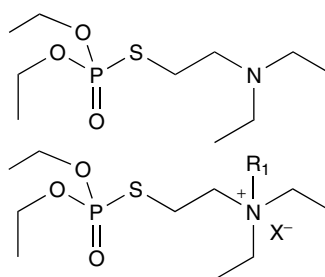
Lewisite 3: Tris(2-chlorovinyl)arsine (40334-70-1)	
(6) Nitrogen mustards: HN1: Bis(2-chloroethyl)ethylamine (538-07-8)	
HN2: Bis(2-chloroethyl)methylamine (51-75-2)	
HN3: Tris(2-chloroethyl)amine (555-77-1)	
(7) Saxitoxin (35523-89-8)	
(8) Ricin (9009-86-3)	Structure not specified
B. Precursors	
(9) Alkyl (R_1 : Me, Et, <i>n</i> -Pr or <i>i</i> -Pr) phosphonyldifluorides e.g. DF: Methylphosphonyldifluoride (676-99-3)	
(10) O-Alkyl (R_1 : H or $\leq C_{10}$, incl. cycloalkyl) O-2-dialkyl ($R_{2,3}$: Me, Et, <i>n</i> -Pr or <i>i</i> -Pr)-aminoethyl alkyl (R_4 : Me, Et, <i>n</i> -Pr or <i>i</i> -Pr) phosphonites and corresponding alkylated or protonated salts (R_5 : alkyl or H; X^- : anion) e.g. QL: O-Ethyl O-2-diisopropylaminoethyl methylphosphonite (57856-11-8)	
	
(11) Chlorosarin: O-isopropyl methylphosphonochloridate (1445-76-7)	
(12) Chlorosoman: O-Pinacolyl methylphosphonochloridate (7040-57-5)	

Schedule 2

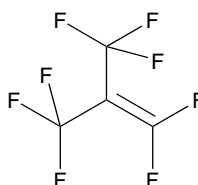
A. Toxic chemicals (CAS reg. No.)

Structure

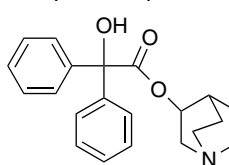
- (1) Amiton: *O,O*-Diethyl *S*-[2-(diethylamino)ethyl] phosphorothiolate (78-53-5) and corresponding alkylated or protonated salts (R_1 : alkyl or H; X^- : anion)



- (2) PFIB: 1,1,3,3,3-Pentafluoro-2-trifluoromethyl-1-propene (382-21-8)

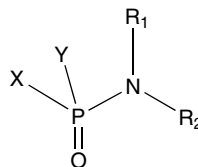


- (3) BZ: 3-Quinuclidinyl benzilate (6581-06-2)*

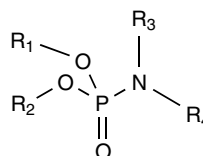


B. Precursors

- (4) Chemicals, except for those listed in Schedule 1, containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms,
e.g. Methylphosphonyl dichloride (676-97-1);
Dimethyl methylphosphonate (756-79-6)
Exemption:
Fonofos: *O*-Ethyl *S*-phenyl ethylphosphonothiothionate (944-22-9)
- (5) *N,N*-Dialkyl ($R_{1,2}$: Me, Et, *n*-Pr or *i*-Pr) phosphoramidic dihalides (X,Y : halogen)



- (6) Dialkyl ($R_{1,2}$: Me, Et, *n*-Pr or *i*-Pr) *N,N*-dialkyl ($R_{3,4}$: Me, Et, *n*-Pr or *i*-Pr)-phosphoramidates

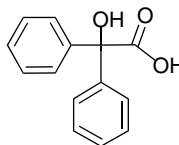


- (7) Arsenic trichloride (7784-34-1)

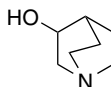


Schedule 2

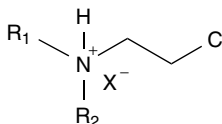
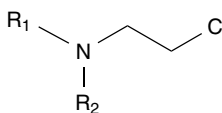
- (8) 2,2-Diphenyl-2-hydroxyacetic acid
(76-93-7)



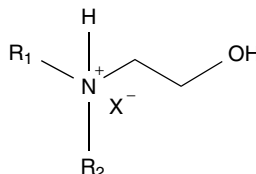
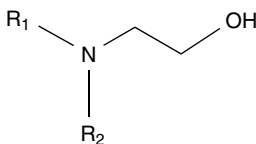
- (9) Quinuclidin-3-ol (1619-34-7)



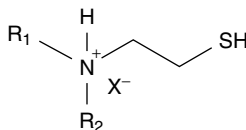
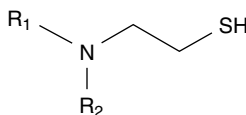
- (10) *N,N*-Dialkyl ($R_{1,2}$: Me, Et, *n*-Pr or *i*-Pr) aminoethyl-2-chlorides and corresponding protonated salts (*X*: anion)



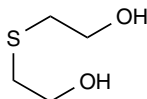
- (11) *N,N*-Dialkyl ($R_{1,2}$: Me, Et, *n*-Pr or *i*-Pr) aminoethane-2-ols and corresponding protonated salts (*X*: anion)
Exemptions:
N,N-Dimethylaminoethanol (108-01-0) and corresponding protonated salts
N,N-Diethylaminoethanol (100-37-8) and corresponding protonated salts



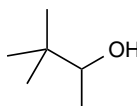
- (12) *N,N*-Dialkyl ($R_{1,2}$: Me, Et, *n*-Pr or *i*-Pr) aminoethane-2-thiols and corresponding protonated salts (*X*: anion)



- (13) Thiodiglycol: Bis(2-hydroxyethyl)sulfide (111-48-8)



- (14) Pinacolyl alcohol: 3,3-Dimethylbutan-2-ol (464-07-3)



Schedule 3	
A. Toxic chemicals (CAS reg. No.)	Structure
(1) <i>Phosgene: Carbonyl dichloride (75-44-5)</i>	COCl_2
(2) Cyanogen chloride (506-77-4)	ClCN
(3) Hydrogen cyanide (74-90-8)	HCN
(4) Chloropicrin: Trichloronitromethane (76-06-2)	Cl_3CNO_2
B. Precursors	
(5) <i>Phosphorus oxychloride (10025-87-3)</i>	POCl_2
(6) Phosphorus trichloride (7719-12-2)	PCl_3
(7) <i>Phosphorus pentachloride (10026-13-8)</i>	PCl_5
(8) Trimethyl phosphite (121-45-9)	$\text{P}(\text{OCH}_3)_3$
(9) Triethyl phosphite (122-52-1)	$\text{P}(\text{OCH}_2\text{CH}_3)_3$
(10) Dimethyl phosphite (868-85-9)	$(\text{CH}_3\text{O})_2\text{POH}$
(11) Diethyl phosphite (762-04-9)	$(\text{CH}_3\text{CH}_2\text{O})_2\text{POH}$
(12) Sulfur monochloride (10025-67-9)	SCl
(13) Sulfur dichloride (10545-99-0)	SCl_2
(14) Thionyl chloride (7719-09-7)	SOCl_2
(15) Ethyldiethanolamine (139-87-7)	$\text{CH}_3\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$
(16) Methyldiethanolamine (105-59-9)	$\text{CH}_3\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$
(17) Triethanolamine (102-71-6)	$\text{N}(\text{CH}_2\text{CH}_2\text{OH})_3$

*Subject to special thresholds for declaration and verification. CAS, Chemical Abstracts Service.

ANNEX 3: EXCERPT FROM DECISION 71 FROM THE FIRST CONFERENCE OF STATES PARTIES

ATTACHMENT 22

PC-XVI/B/WP.6, ANNEX 6

ANNEX 6

Technical Specifications for GC/MS Sample Preparation Kit

The contents of the GC/MS sample preparation kit are based on the joint procedures provided by Finland and the United States of America. The application of these procedures resulted in the sample preparation kit consisting of the items, sufficient for the analysis of a maximum of four soils and a total

of nine aqueous/wipe samples. This kit should also allow the processing of other materials, bulk liquid, and vapour/aerosol samples (taken with the sampling kit, PC-VIII/B/WP.12, Annex 4), although studies with these sample types have not been done. If a larger number of samples of a single type is expected to be taken, the contents of the kit must be modified accordingly. These items will have to be packaged in suitable shipping containers.

GC/MS Sample Preparation Kit

Reusable items	Minimum number
Fume hood fulfilling the present internationally recognized requirements set for fume hoods, such as DIN, OSHA (US) and BS standards. Normal linear airflow velocity at the face should be over 0.5 m/s (measured in compliance with BSI DD80 or equivalent) with approx. size 90 × 90 × 90 cm.	1
Nitrogen generator (or equivalent cylinder with regulator) for heater/evaporator	1
A heater/evaporator with separate aluminium heating blocks (three sizes compatible with the <i>vials glassware</i>) and <i>6-position nitrogen purge concentrator with tubing for connection to nitrogen generator</i>	1
Top loading balance, electronic, with capacity 0–200 g, precision ± 0.1 g	1
Centrifugal evaporator, with 4 to 8 sample rotor compatible with <i>25 ml glass centrifuge tube glassware</i>	1
Rotorvane pump with water trap for centrifugal evaporator	1
SPE 12 port vacuum manifold with vial holder compatible with <i>the vials glassware</i>	1
Vacuum and pressure station for SPE <i>12 port vacuum manifold</i>	1
<i>Trap kit for SPE 12 port vacuum manifold</i>	1
Adjustable pipette, 0.5–5 ml	2
<i>Adjustable pipette, 2–10 ml</i>	2
Bar-code reader	1
<i>Centrifuge compatible with 15 ml polypropylene centrifuge tube</i>	1
Tube rack compatible with centrifuge tubes	1
<i>Tube rack compatible with the vials, glass filled acetal</i>	1
<i>Digital alarm timer</i>	1
Disposable items¹	Minimum number
Beaker, glass, 50 ml	6
Glass vial with screw cap, Teflon liner, 8 ml (<i>approximately</i>)	20
Glass vial with screw cap, Teflon liner, 25 ml (<i>approximately</i>)	144 (100)
<i>Glass vial with screw cap, Teflon liner, 4 ml (approximately)</i>	144
<i>Glass insert compatible with 4 ml glass vial, 0.35 ml (approximately)</i>	20
Graduated cylinder, glass, 10 ml	20
Pipette tip, for adjustable pipette (0.5–5 ml)	40
<i>Pipette tip, for adjustable pipette (2–10 ml)</i>	40
Syringe, <i>polypropylene glass</i> , luer lock, 10 ml	100 (20)
<i>Syringe filter, polypropylene, 25 mm diameter, 0.45 micrometer pore size with binder-free glass fiber prefilter</i>	100
Syringe filter, PTFE, 25 mm diameter, 1.0 micrometer pore size	20
Syringe filter, PTFE, 25 mm diameter, 0.45 micrometer pore size	20
Syringe filter, LC, 25 mm diameter, 0.45 micrometer pore size	10
<i>Sterilization filter unit, nylon, 200 ml, 0.45 micrometer pore size</i>	20
Sterilization Filter Unit cellulose acetate, 0.45 micrometer	20
Sterilization Filter Unit cellulose nitrate, 0.45 micrometer	20

(continued overleaf)

(continued)

Reusable items	Minimum number
Syringe, glass, 500 <i>microliter</i> ml	6
Filter paper, Whatman number 4, diameter 90 mm for funnels	1 pack
Funnel, short stem, diameter 5 cm	50 8
SPE Cartridge, SCX SAX , 500 mg/3 ml	20
SPE Amino Cartridge, NH₂100 mg/1 ml	20
Adapter for SPE Cartridge, <i>compatible with SCX/NH₂ cartridge and 10 ml polypropylene syringe</i>	40 (20)
Outlet needles for SPE 12 port vacuum manifold (SPE-outlet-needles)	24
Spare stopcocks for SPE 12 port vacuum manifold	12
Stainless steel needles compatible with 6-position nitrogen purge concentrator	6
Replacement tubing for 6-position nitrogen purge concentrator	1 m
Hose clamps compatible with the tubing for 6-position nitrogen purge concentrator	4
Centrifuge tube, glass, 25 ml	50 (20)
Centrifuge tube with plug seal cap, polypropylene, 15 ml, rated to 3000 G	50
Transfer pipette bulbs, rubber	1 pack
Transfer pipette, <i>glass</i> , short neck, 2 ml	1 pack
Dichloromethane, gas chromatographic grade	500 ml
Water, type 1 (ASTM)	100 ml
Methanol – Triethylamine (1 %, v/v)	200 ml
<i>N,O</i> -bis-(trimethylsilyl)trifluoroacetamide (BSTFA)	20 ml
Hexane, gas chromatographic grade	25 ml
0.1 N Hydrochloric acid, HCl, reagent grade	50 ml
2.0 N Hydrochloric acid, HCl, reagent grade	50 ml
0.1 N Ammonium hydroxide, NH ₄ OH, reagent grade	50 ml
Ammonium hydroxide, NH₄OH, 27–30 % as Ammonia, reagent grade	25 ml
Disposable items (continued)	Minimum number
Methanol, gas chromatographic grade, 99.8 %	500 ml
pH paper, 1–14	1 roll
pH paper, 9 to 12 in increments of 0.5 pH units	1 roll
pH paper, 6.8 to 8.4 in increments of 0.5 pH units	1 roll
pH paper, 3.0 to 5.5 in increments of 0.5 pH units	1 roll
pH paper, 0.0 to 3.0 in increments of 0.5 pH units	1 roll
Sodium sulfate, anhydrous, Na ₂ SO ₄ , reagent grade	50 g
Decontamination solution, site dependent	2 liters
Tetrahydrofuran (THF), anhydrous, 99.9 % pure, inhibitor free, <i>stabilised with less than 0.025 % Butylated hydroxy toluene</i>	100 ml
3,4-Dimercaptotoluene(DMT) 3,4-dithiotoluene	1 ml 5 g
Hexachlorobenzene 50 ng/ml (ng/microliter) in dichloromethane	2 ml
Test mixture solution containing 12.5 40 µg ml ⁻¹ (microgram per milliliter) <i>each</i> in dichloromethane of:	2 ml
Trimethylphosphate	
2,6-Dimethylphenol	
5-Chloro-2-methylaniline	
Tri- <i>n</i> -butylphosphate	

(continued)

Reusable items	Minimum number
Dibenzothiophene	
Malathion	
Methylstearate	
<i>Alkane mixture</i> Test solution containing <i>n</i> -alkanes (C ₈ – C ₂₄ , even members numbers) at a concentration of 50 40 µg/ml (microgram per milliliter)	2 ml
Bar-code label (with also alphanumeric readout)	1 pack
<i>Adhesive label, 8 mm × 20 mm (approximately)</i>	1 pack
Sample preparation form, carbonless, duplicate	1 set
Clipboard for sample preparation form	1
<i>Laboratory notebook</i>	1
Pen, permanent, archive approved	2
Marker, permanent	4
Pair of scissors	1
Forceps, stainless steel	5
Sealing film	1 pack
Spatula, stainless steel, spoon type	12 4
Absorbent wipe, standard	2 boxes
Laboratory coat	2 coats
Gloves, latex	2 boxes 1-pack
Gloves, durable, chemically resistant	1 pack
Disposable items (continued)	Minimum number
Waste bottle, wide mouth, volume 2 liters with chemical resistant lid	1
Waste bottle, wide mouth, volume 4 liters with chemical resistant lid	1
<i>Wash bottle, polyethylene, 250 ml</i>	2

¹These items are replacement components used to replenish the kits.**~~Bold~~** = Deleted from the original list; and*Italics* = Added to the original list.

CHAPTER 3

On-site Analysis by the Inspection Team. Sampling, Analysis, Equipment, Procedures and Strategies

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1 INTRODUCTION

The Chemical Weapons Convention (CWC) provides sampling and analysis as a tool for verification. General procedures to use sampling and analysis are outlined under the general rules of verification in the Verification Annex (VA) Part II paragraph 52–58, special provisions concerning different types of inspections are given in various parts of the VA.

On the basis of this framework from the CWC, the Technical Secretariat and the OPCW Laboratory have built a complex network of linked procedures for sampling and analysis and related activities. Experience gathered during inspections, training, exercises, and proficiency tests led to improvement

of existing methods and development of new ones. Equipment has been tested under laboratory and field conditions and – sometimes in close co-operation with a supplier – improvements have been achieved. Approved Standard Operating Procedures and Work Instructions, which are part of the OPCW quality system, describe operations, methods, and equipment used for on-site sampling and analysis ⁽¹⁾.

The following article will take the reader through the various phases of an inspection, from mission planning to the return of the inspection team to the OPCW headquarters. By this, it will describe the concept of sampling and analysis, the equipment and procedures used during inspections, highlight some critical aspects, and refer to experiences gained.

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

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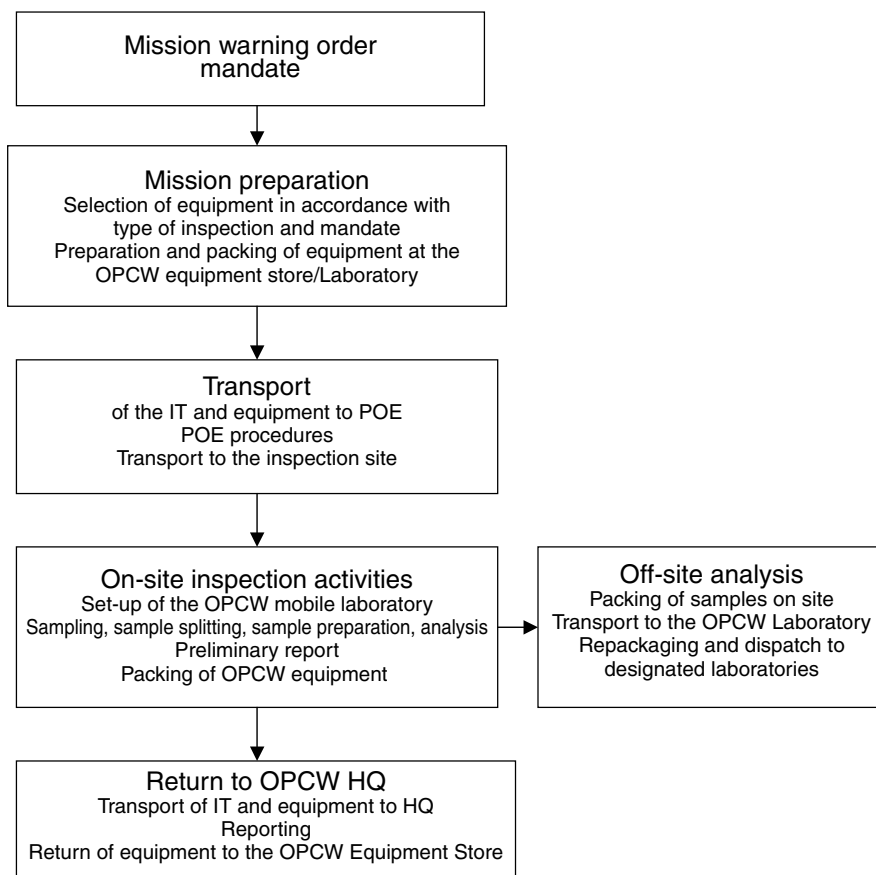


Figure 1. Schematic order of inspection activities related to sampling and analysis

The general order of inspection-related activities is given in Figure 1.

2 PREPARATION FOR AN INSPECTION

2.1 Equipment

Preparation for an inspection starts when the Inspection Team Leader (ITL) receives a ‘mission warning order’, which provides details of type and duration of the inspection mission and the names of the team members. In preparation of this mission, the Inspection Team (IT) selects the necessary equipment from the list of equipment approved by the First

Conference of State Parties ⁽²⁾. In regard to sampling and analysis (S&A), the equipment currently available is

- Sample collection kit
- Sample preparation kit
- Sample transport kit (for off-site sample analysis)
- Analytical instruments: GC/MS
- Supporting equipment.

The equipment is selected on the basis of the type of mission to be conducted and the requirements resulting from the ‘Inspection Mandate’, which contains the instructions issued by the Director-General to the inspection team for the conduct of a particular inspection. For an Investigation of Alleged Use (IAU) or a Challenge Inspection (CI), the complete S&A equipment will be taken to the site,

while for Chemical Weapons Destruction Facility (CWDF) or Schedule 2 facility inspections, only some items may be selected. Details in regard to different types of inspection provided for in the CWC and their specific requirements in relation to S&A as well as information on the list of approved equipment can be found in **Chapter 2**.

2.1.1 The Sample Collection Kit

The kit contains over 60 different items of equipment, some of which are optional. The items are packed in three rigid transport boxes in separate compartments and drawers embedded in form adapted foam cut-outs. The weight of the complete packed kit is ~120 kg. The type of packaging has been developed on the basis of experiences gained during inspections and exercises (*see picture in Chapter 2*).

Items in the sample collection kit are listed in Table 1:

A prepackaged kit for sampling of water and liquid contains a syringe connected by a three-way Luer lock connector to Teflon tubing with a weight on the end of the tubing. In addition prepacked 'Vacutainer' kits are available where an evacuated tube (as normally used for blood sampling) can be utilized to collect liquids with a low viscosity from storage containers, drums, reactors, and so on. The material of items that may come in contact with a sample is glass, stainless steel, or Teflon (with exemption of 60 mL polyethylene syringes used for the collection of water samples). All items that may come in direct contact with a sample are brought on site in sealed packaging that will be opened directly before sampling in order to prevent environmental contamination.

The amount of material provided in a complete sample collection kit is sufficient to collect eight samples of each type (bulk solid, soil, water, liquid, and wipe). The perception of the sample collection kit is mainly aimed at environmental samples to be taken by the OPCW inspectors as expected in IAU inspections or perimeter monitoring at CI inspections. Nevertheless, it covers sampling activities at industrial sites, where samples are most likely to be taken from storage devices, production equipment, and waste streams.

An additional part of the sample collection kit are solvents (dichloromethane, methanol, water) and decontamination solutions. Solvents are necessary – in accordance with approved procedures – to collect wipe samples. These solvents need to be transported as dangerous goods, which require special transport arrangements.

Optional items of the sample collection kit are tools necessary to collect air samples. Even though these items are approved equipment, they have not been used so far because of the absence of approved procedures for sampling, sample splitting, and analysis (using thermal desorption). Methods for air monitoring have been developed and validated by VERIFIN⁽³⁾ using analytical methods not available at OPCW on-site laboratories. For verification purposes, air sampling has not yet been proven to be of high priority. Instead, sampling of the sources of air contamination is considered to be more valuable.

Continuous air sampling (monitoring) for health and safety purposes is the responsibility of the ISP (Inspected State Party). For real-time monitoring over a short period, the IT has the option to use handheld detectors triggering an alarm if contamination levels exceed a certain concentration. In case of

Table 1. Material provided in the sample collection kit

-
1. Preassembled kits for sampling of water or organic liquid, separately packed and sealed
 2. Scoops, bowl, spatulas for collection of soil samples
 3. Separately packed wipes, stiff wires, alligator clips, clamps to take wipe samples
 4. Vials and glass bottles, different sizes, separately packed and sealed, to hold the samples
 5. Spare items for sampling of water or liquid samples, i.e. spare syringes, vacutainers, tubing
 6. Pipettes and pipette balls used for sample splitting
 7. Bags, tape, and seals to package sample vials or bottles
 8. 'Peli' cases to transport the sampling equipment to the sampling point
 9. General items: gloves, goggles, pens, knife, scissors, labels, markers, ground sheets, garbage bags
-

absence of suitable detection capabilities, inspectors will wear adequate personal protective equipment.

2.1.2 The Sample Preparation Kit

Like the items for sample collection, the more than 50 different items of equipment for sample preparation are packed in four transport boxes (weight ~ 170 kg) in shape-fitted compartments or drawers (see the picture in **Chapter 2**). The sample preparation equipment is tailored to the approved sample preparation procedures (see Section 3.4).

Items packed in the sample preparation kit are listed in Table 2:

In addition, a portable fume hood (3 boxes), a portable refrigerator and an on-site sample transport

container (1 box), and chemicals necessary for sample preparation are part of the sample preparation kit.

The amount of material provided in the sample preparation kit is supposed to be sufficient to perform sample splitting and sample preparation on site for several samples of each matrix. Laboratory glassware is brought on site in sealed packaging to assure cleanness before use. Supporting material (filter paper, SPE (Solid Phase Extraction) cartridges) is carried in the original packaging from the supplier. The sample preparation kit includes various chemicals necessary to perform sample preparation procedures as well as test mixture and internal standard solution for GC/MS (Gas Chromatograph/Mass Spectrometer) analysis. Only

Table 2. Material and chemicals provided in the sample preparation kit

1. Centrifugal evaporator with vacuum pump
2. Heater/evaporator to be used with pure nitrogen from a nitrogen generator (optional item) or a nitrogen cylinder
3. Basic laboratory equipment, i.e. balance, centrifuge, ultrasonic bath
4. Laboratory glassware, i.e. vials, tubes, funnels, beakers, pipettes, syringes, measuring cylinders
5. Supporting equipment, i.e. trays, filter paper, pH paper, SPE cartridges, adjustable pipettes and pipette tips, labels, markers, scissors, bench coat paper, containers and bags for waste collection, laboratory coats, gloves, stationary
6. Laboratory fume hood
7. Portable refrigerator, on-site sample transport container
8. Chemicals and solvents for sample preparation and GC/MS analysis:
Dichloromethane, GC grade
Methanol, GC grade
Hexane, GC grade
Water, HPLC ^a grade
Tetrahydrofuran 99 %, stabilized with 0.025 % butylated hydroxy toluene (THF) ^b
2 % (v/v) Hydrochloric acid in methanol
0.1 N Hydrochloric acid, reagent grade
2 N Hydrochloric acid, reagent grade
0.1 N Ammonium hydroxide, reagent grade
1 % (v/v) Triethylamine in methanol
Sodium sulfate, anhydrous, reagent grade
<i>N,O</i> -bis-(Trimethylsilyl)trifluoroacetamide (BSTFA)
3,4-Dimercaptotoluene in acetone, 5 mg/mL freshly prepared (DMT)
OPCW GC/MS TEST MIXTURE containing in dichloromethane 10 µg/mL of
<i>n</i> -Alkanes C ₈ –C ₂₄ even numbers
Trimethylphosphate
2,6-Dimethylphenol
5-Chloro-2-methylaniline
Tri- <i>n</i> -butylphosphate
Dibenzothiophene
Malathion
Methylstearate
OPCW GC/MS HCB mixture containing 50 µg/mL of hexachlorobenzene in dichloromethane

^aHPLC – High Performance Liquid Chromatography

^bTHF – Tetrahydrofuran

unopened containers as delivered by the supplier are brought on site; standards have a certificate of conformity from the supplier. None of the chemicals is a scheduled chemical or a direct degradation product thereof. The chemicals for sample preparation need to be transported as dangerous goods, which implies that packing is to be performed by personnel certified for packing of dangerous goods. These chemicals are excluded from transport on passenger aircraft and must therefore be sent separately from the inspection team by cargo.

2.1.3 Sample Transport Kits

Sample transport kits are designed to fulfil the requirements for air transport of OPCW samples for off-site analysis under International Air Transport Association (IATA) provision 106 and international standards for road, railway, and sea transport.

OPCW equipment includes two different types of sample transport kits: (see the pictures in **Chapter 2**).

- A small-sample transport container that may hold a maximum of 10 g of pure material or 400 g of diluted material or 8 air sampling tubes each of which may have adsorbed 10 mg. The outer transport packing is a wooden box with a size of 32 × 40 × 33 cm, its weight approximately 30 kg.
- A large-sample transport container that may hold a maximum of 350 mL of any material. The outer transport packing of the kit is a conventional 200 L steel barrel, the weight of the kit is approximately 85 kg.

A detailed description of the kits together with the packing procedures is given in Section 3.6

2.1.4 Analytical Instruments

The analytical instrument currently used in the OPCW mobile laboratory is the portable GC/MS Bruker EM 640S. The instrument and supplementary equipment are packed in 5 transport boxes. (The instrument and procedures for use are described in **Chapter 2** and **Chapter 4**) Before being issued for an inspection, the instrument is validated for on-site use at the OPCW Laboratory following

OPCW procedures. After validation and packing has been completed, the Office of Internal Oversight (OIO) audits the process of instrument preparation and issues a certificate for on-site use. The OPCW Laboratory and the OIO are accredited for this process. The instrument is to be operated exclusively by AC inspectors who are trained and authorized for this task. At least one of the ACs participating in the inspection will assist during the instrument validation and packing process at the OPCW Laboratory.

2.1.5 Supporting Equipment

Items of the approved equipment that support S&A activities are

- individual protective equipment (e.g. gloves, protective goggles, coverall, mask with canister, air permeable suit, boots, chemical resistant suit, SCBA (self containing breathing apparatus));
- team decontamination equipment;
- detectors (i.e. hand held detectors, military detector kit);
- power generator (for laboratory power supply).

This equipment is general team equipment and can be used during inspection for various activities.

2.2 Packing and Transport

All OPCW equipment is stored, maintained, and packed in the OPCW equipment store located (together with the OPCW Laboratory) in Rijswijk. As soon as the IT has decided which items of approved equipment will be taken for its mission, packing can commence in the equipment store. At least one IT member will check the functioning of equipment and completeness of packing for each item before its transport case is finally closed and sealed. If the inspection equipment includes items for sampling and analysis, one inspector analytical chemist will assist in the equipment check.

Operations and Planning Branch will arrange for the transport of equipment to the Point of Entry (POE) of the inspected State Party. While the small

amount of equipment necessary for routine industrial inspections and CWDF rotations may be hand-carried by the IT, large items of the OPCW mobile laboratory and dangerous goods are sent as cargo. In case of a Challenge Inspection or an Investigation of Alleged Use it may be necessary to charter an aircraft to transport the IT and the equipment together.

2.3 POE Procedures

In accordance with CWC VA Part II, paragraph 29, the ISP has the right to inspect approved equipment in the presence of inspection team members at the Point of Entry (POE). The POE is a place designated by the State Party as the entry point for the inspection team to its territory; it is normally an airport or a border checkpoint. Details for POE procedures are described in ⁽⁴⁾. The equipment check at the POE may include visual inspection and nondestructive tests, that is, the check of the identity of the equipment, the technical specifications, conformity with operational requirements, certificates, and the authentication documents for each item. POE procedures may include inspection of software and documentation stored on computer hard drives. POE procedures must not interfere in any way with the functionality of the equipment or render it inoperable. The ISP has the right to exclude equipment that does not meet the requirements of approved equipment or lacks the authentication documents. On the basis of five years experience, POE procedures range from a check of the packing lists provided with the equipment to thorough inspection of each item including functionality testing, X-ray examination, and CW (Chemical Weapons) agent monitoring. In some cases, a simple typing error in a serial number has been the reason for refusal of an item. Concerning specialized equipment for sampling and analysis, it is the practice to check items for their identity at the POE but to demonstrate the functionality, for example, of a GC/MS instrument, after its installation in the OPCW on-site laboratory.

Once POE procedures are completed, the inspection team and the equipment are transported to the inspection site.

3 ON-SITE INSPECTION PERIOD

3.1 Setting Up the OPCW Mobile Laboratory

The OPCW mobile laboratory has been set up on inspection, training and exercises in various locations and under different conditions. On the basis of the experience gained, the room to set up the complete OPCW mobile laboratory including all equipment for sample preparation and analysis should fulfil the following minimum requirements:

- approximate size 20 m², ceiling height 2 m;
- lights and electrical power (220 V/50 Hz/25 A or 110 V/60 Hz/50 A);
- a window or suitable opening to install the fume hood outlet;
- 1 large sturdy table to carry the fume hood (~250 kg, 90 × 90 cm surface);
- 1 large sturdy table to carry the GC/MS (~100 kg, 100 × 60 cm surface);
- 2 tables with chairs for working and writing;
- room temperature between 15 and 25 °C.

The laboratory can be set up in approximately three hours by three inspectors. The set-up of the 'portable' fume hood due to its size and weight is most time consuming and requires physical strength. The set-up of the GC/MS instrument and its preparation need approximately two hours and can be done by one person. However, lifting the instrument



Picture 1. OPCW mobile laboratory set up during an IAU exercise

body out of the transport case requires assistance. It has been repeatedly demonstrated that the complete OPCW mobile laboratory equipment is fully operational within four hours.

3.2 Sample Collection

3.2.1 Types of Samples

The types of samples to be collected depend on the type of inspection being conducted, the facility being inspected, and the particular verification aims to be achieved. Among other things, the following samples may be collected in inspections as listed below:

Chemical Weapons Storage Facilities (CWSF):

- tagging of munitions and containers for later sampling at the CWSF or CWDF.

Chemical Weapons Destruction Facilities (CWDF):

- neat agent or bulk chemical samples from various types of munitions and storage devices to verify the type of declared chemical that is destroyed;
- samples of end products such as brine solution, ashes, slag, waste water, gaseous effluents to verify the completeness of destruction.

Declared Schedule 1, 2, and 3 facilities:

- neat chemicals, process, and waste samples to verify the presence of declared chemicals and the absence of undeclared chemicals.

Challenge Inspection (CI):

- neat chemicals, process, and waste samples to verify the absence of undeclared chemicals;
- environmental samples (wipe, air, soil, or effluent) at the perimeter before access to the facility is provided.

Investigation of Alleged Use (IAU):

- agent samples and degradation products from exploded and unexploded munitions;
- environmental samples from the area of alleged use;
- used decontamination solution;

- any type of material and wipe samples from any surface that might have been contaminated.

Samples may be of solid or liquid matrix or be a surface wipe sample from various sampling points as indicated below:

Solid samples can consist of materials such as bulk chemicals, soil, paints, plastics, wood, rubber, garment, packing and filter material. Sampling points can be, for example,

- contents of containers;
- gaskets of valves and reactors;
- inner walls of storage areas, production or synthesis laboratories, and fume hoods;
- filters;
- exhaust pipes;
- any absorbent material such as wood, concrete, paint, clothing, or garment;
- soil in or around suspected sites.

Liquid samples for example can be taken from

- liquid chemicals or mixtures in drums, reservoirs, reactor vessels, pipes, tanks;
- waste effluents;
- spillage near chemical containers, under pipelines (possible leakage during storage or operation);
- used decontamination solutions;
- chemical dumps and sewage.

Liquid samples can consist of organic liquids, aqueous solutions, or water. Environmental water samples can be taken from water surface down to a depth of five meters, from (rain) pools, lakes, rivers, waste effluents, or sewage.

If no apparent liquid/solid samples are available *wipe samples* can be collected from various surfaces, for example,

- the inside of reactor vessels, storage tanks, containers;
- around valves and flanges;
- from leaks and spills on a floor or wall;
- inside fume hoods, openings of ventilation systems;
- from munitions fragments, contaminated equipment.

Adsorbent cotton is used as a wipe material. Dichloromethane or methanol can be used as wetting solvent.

Whenever possible, a *background sample* of similar composition as the authentic sample is collected. For background sampling, the same type of sample collection equipment and techniques are applied as that used while collecting the authentic sample(s). Representative background samples will help to determine whether there are matrix effects, which may interfere in the sample preparation and analysis. Background and blank samples are required to determine whether the environment, sampling equipment, sample preparation, and analysis procedures alter the analytes of interest in the sample or interfere with the analysis.

Aqueous background samples are collected from similar sources (i.e. upstream of effluent output, puddles of water, tap water, etc.) outside the possibly contaminated area. If a background aqueous sample cannot be collected, tap water or deionized/distilled water can be used as a background sample.

Usually, a representative background sample cannot be collected for bulk, neat, or dilute organic solids and liquids. In these cases, a *blank sample* consisting of a sampling vial filled with dichloromethane is used. If the equipment or chemicals provided by the ISP have been used for sample collection they also will be used for the corresponding background sample(s).

In order to have sufficient sample material for on-site and off-site analysis, OPCW procedures recommend collecting the amount of sample material listed in Table 3:

Table 3. Recommended amounts of samples

Type of sample	Amount recommended
Solid bulk chemical	<10 g
Soil	250 g
Paint, rubber, wood etc.	Surface scratch of 5 × 5 cm
Organic liquid, non-CW	5 ml
Organic liquid, CW or highly toxic	<1 ml
Aqueous	250 ml
Wipe	1 wipe per wetting solvent

3.2.2 Sampling Procedures

The CWC stipulates that samples are collected by ISP or facility representatives at the request of the inspection team in the presence of inspectors. If so agreed in advance, the inspection team may take samples by itself (CWC VA Part II Paragraph 52).

For Investigation of Alleged Use, the CWC states that the inspection team shall have the right to collect samples of types and in quantities considered necessary; if so requested, the ISP shall assist in the collection of samples (CWC VA Part XI Paragraph 16).

OPCW procedures describe the collection of samples for all types of inspections in several work instructions ⁽¹⁾. For each sampling operation, it is crucial that the analytical chemists of the inspection team collect all available information on the chemicals involved, processes used, reaction mechanisms, possible by-products, and so on. This information is necessary to determine the appropriate sampling and sample preparation procedures and later to evaluate analytical results.

Before taking a sample, the sampling area or point has to be inspected by IT members in order to

- determine the degree of contamination and other hazards;
- locate and mark (if necessary) the sampling point(s);
- assess the number and type of samples to be taken;
- determine the amount to be taken;
- determine the equipment required;
- take pictures, if possible.

As a rule OPCW equipment is used for sample collection and handed to the ISP/facility representative who will perform the sampling activity. If ISP equipment is used for sample collection, the IT will prepare a respective equipment blank sample. Sampling can be observed either directly by an inspector or from an on-site close circuit television system. The sampling operation and subsequent chain of custody procedures must be well established between the ISP and the IT before activities commence.

The level of IPE (individual protective equipment) required would depend on the type of sample being collected and the respective hazards involved. It may

range from a laboratory coat to a fully encapsulated suit.

The Technical Secretariat together with States Parties has conducted several IAU exercises including sampling under hazardous conditions and analysis of toxic samples. During these exercises, a reconnaissance team dressed in protective equipment evaluated the area where chemical weapons allegedly had been used. Munitions specialists in this team established secure ways to the possible sampling points. The reconnaissance team took notes, photographs, and video records, and marked selected sampling points. Dedicated sampling teams consisting of at least four persons dressed in protective equipment performed the actual sampling (see picture 2). The sampling team included one person of the reconnaissance team; the team leader was an analytical chemist. Sampling equipment was selected from the sample collection kit and hand carried in on-site transport ('pelli') cases to the sampling area. One important lesson learned from IAU exercises was that the sampling points must be well chosen because

- Preparation of sampling equipment and dressing of protective equipment followed by the sampling operation is time consuming. The sampling team has only limited time to reach the sampling points and perform sampling, when dressed in protective suits, that is, at temperatures below 25 °C, about 2 hours; when using SCBA equipment about 45 minutes.
- The amount of sampling equipment that can be brought into the sampling area is limited; therefore, a small number of samples should be taken as close as possible to a potential source rather than trying to screen a large area by collecting many samples.
- The number of samples that can be analyzed in the on-site laboratory within the time lines set by the CWC is primarily limited by the time required for GC/MS analysis.

If decontamination ('hotline') procedures are required, the containers with the samples will leave the contaminated area only after decontamination is performed. All disposable items for sample collection will be left inside the 'hot' area. Blank samples of soil or water are taken (at presumably not contaminated points) outside the 'hot' area.



Picture 2. OPCW sampling team during an IAU exercise

After sampling (and decontamination, if required), the lid of the sample container is secured by tape to prevent it from opening because of vibrations during transport. The sample container is then sealed with one or two OPCW frangible, tamper-proof seals placed over the lid, the tape, and the container body. These seals have unique numbers, which also serve as an identifier for the sample. When the seal is removed, it is irreversibly damaged.

3.3 Sample Splitting

The ISP has the right to retain portions of all samples taken or to take duplicate samples (CWC VA Part II paragraph 54); therefore, any sample collected during an inspection is split into several fractions. In case of off-site analysis, split portions of the respective sample are sent to a minimum of two designated laboratories. These split portions are prepared, packed, and sealed on site during the inspection and are only opened at designated laboratories.

OPCW procedures require sample splitting into eight portions, which are distributed as follows:

- two portions for on-site analysis by the inspection team;
- one portion for the ISP;
- one portion to be kept as a reference on site under joint OPCW and ISP seal;
- four portions to be sent for off-site analysis.

This splitting pattern implies that enough sample material needs to be sampled in order to prepare split

portions large enough to perform sample preparation as required (see Table 3).

Sample material must be mixed thoroughly before splitting to assure equal distribution of analytes in each split portion. While mixing is easily achieved for liquid samples, it is problematic for solid samples and impossible for wipe samples. In case of a wipe sample, the problem can be overcome by extracting the wipe with a hydrophobic/nonpolar solvent and afterwards a hydrophilic/polar solvent, for example, dichloromethane and water, and split the extracts. The same method may be applied to samples such as garment, rubber, wood, filter material, or paint. Soil samples should be mixed in a bowl (provided in the sample collection kit) directly after sampling before filling into the sample container. For splitting, the cone and quarter method is recommended, but depending on the type and consistence of the soil, may not always be possible to perform.

Sample splitting is performed at the OPCW on-site laboratory (if available) except for highly toxic samples, which require decontamination and hot line procedures. These samples (e.g. neat CW agent) are split directly after sampling into eight small sub-samples. This reduces the risk of contamination of the on-site laboratory, especially the fume hood. Afterwards, only one split sample of approximately 100 μ L (see Table 3) will be handled in the fume hood for sample preparation.

An alternative to the splitting procedures described above, if agreed in advance with the ISP, is to take two equal portions sufficient for analysis out of the original sample. Of the two portions, one will be used for on-site analysis and the other one is handed to the ISP. If after on-site analysis no further processing is required, the remaining sample can be returned to the ISP or destroyed. In case off-site analysis, the remaining sample will then be divided into six equal portions.

3.4 Sample Preparation

Methods of sample preparation depend on the type of target compounds and the type of analysis to be performed. Because the chemicals related to the CWC include a large variety of organic compounds, OPCW sample preparation procedures are generic using solid–liquid and liquid–liquid extraction. The objective of sample preparation is to

extract analytes of interest from the sample matrix making them suitable for GC/MS analysis, using derivatization if necessary. Nonpolar compounds are recovered by dichloromethane extraction. Polar compounds are recovered from aqueous solutions by evaporation and subsequently analyzed after derivatization of the residue with silylation reagent *N,O*-bis-(Trimethylsilyl)trifluoroacetamide (BSTFA). Lewisite 1 and lewisite 1 oxides are recovered as their dimercaptotoluene (DMT) derivatives.

The general scheme of sample preparation for GC/MS analysis is given in Figure 2.

Sample preparation takes 30 minutes to one hour for dichloromethane extracts and up to six hours for evaporation and derivatization of aqueous solutions. The corresponding background sample or blank is always prepared together with the authentic sample.

3.5 Sample Analysis with GC/MS

The normal sequence for GC/MS analysis of samples includes a consequent series of steps; after each step, the outcome is evaluated to ensure that the results fulfil the criteria described in OPCW procedures before proceeding with the next step (for details *see Chapter 4*). The recommended sequence of analysis includes

- Performance test and retention index calibration at the beginning and end of a working day (or at least once per day) with OPCW test mixture.
- Analysis of solvent or reagent blank co-injected with an internal standard.
- Analysis of the background or blank sample co-injected with an internal standard.
- Analysis of the authentic sample co-injected with an internal standard.

The performance of GC and MS is tested and retention indices are calibrated by injection of the OPCW test mixture. This mixture is a solution of nine *n*-alkanes with even carbon numbers (C_8 to C_{24}) and seven test compounds (nonscheduled chemicals) in dichloromethane (for details *see Chapter 4*).

Hexachlorobenzene (HCB) is co-injected as an internal standard for each analysis as a quality control measure. The detection of the internal standard confirms that the sample was injected

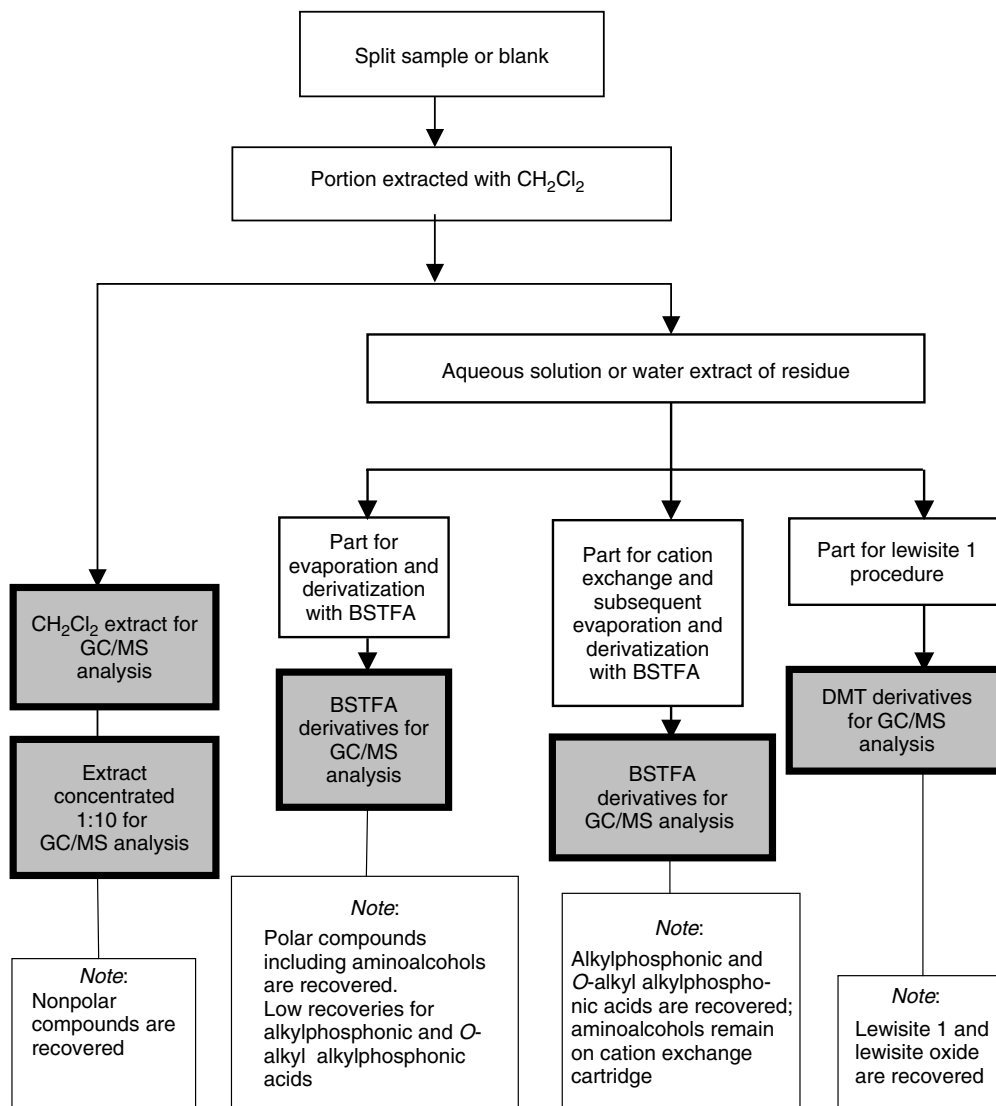


Figure 2. General scheme of sample preparation for GC/MS analysis

successfully and that the instrument was working properly. This is particularly required when the instrument is operated in 'blinded' mode (see below), where the chromatogram is not displayed during or after the analysis. If after step (d) (sample analysis) another sample preparation is analyzed, steps (b) to (d) or at least (c) and (d) are repeated. The temperature programmed GC/MS analysis method has a run time of 45 minutes (including the time to cool down the GC oven). The full

sequence of analysis for one sample preparation and corresponding background/blank requires therefore 1.5 to 2 hours. Taking into account that an authentic sample and its blanks yield eight to ten separate sample preparations, if all steps in Figure 2 are performed, a complete analysis for one sample cannot be performed in less than 7 hours. Analysis time can be shortened, if owing to other information, certain sample preparation steps can be omitted because analysis for

particular compounds is not required (e.g. lewisite procedures).

Extensive technical measures have been implemented to allow the ISP to protect confidential, commercial, or sensible military information during on-site analysis. These measures include in particular the option of ‘blinding’ the GC/MS operating software and ‘security level filters’ of AMDIS (Automated Mass Spectral Deconvolution and Identification System), the GC/MS raw data processing software. These measures can be applied separately or combined, offering the ISP the choice of a gradual restriction of the information revealed to the IT (for details *see* **Chapter 2**).

3.6 Sending Samples Off-site

While the CWC stipulates that samples should preferably be analyzed on site (VA Part II Paragraph 53), the inspection team shall, if it deems necessary, transfer samples for analysis off site at designated laboratories (VA Part II Paragraph 55). The necessity to send samples for off-site analysis may arise from various reasons, such as

- positive identification of undeclared Scheduled chemicals, not explained by the ISP;
- lack of capability for on-site analysis due to absence of an on-site laboratory;
- lack of appropriate equipment and/or procedures for sample preparation and/or analysis capability due to difficult matrices, equipment malfunction, and so on;
- analysis results obtained are unclear;
- the ISP requests off-site analysis for confirmation or clarification.

The decision to send samples off site is made by the ITL in consultation with the ACs of the inspection team. The ITL will send a request to the Director-General, asking permission to send samples for off-site analysis. The approval of this request will trigger various activities at the HQ and on site.

HQ activities include

- invoking of an approval to transport samples under IATA regulation A106 (see below);
- arrangement of transport for the sample(s) and an OPCW courier from the Point of Exit to the OPCW Laboratory in Rijswijk;

- selection of at least two designated laboratories to perform sample analysis;
- notification of these laboratories and their national authorities, receipt of confirmation;
- information to the ISP concerning arrangements for their representatives to observe activities (if so requested) at the OPCW Laboratory and/or the designated laboratories;
- transport arrangements for samples and an OPCW representative from the OPCW Laboratory to the designated laboratories;
- preparation of control samples and blanks at the OPCW Laboratory (see below).

On-site activities include

- preparation and packing of 4–5 split samples for transport to the OPCW Laboratory (packing must be performed by an IT member who is a certified Dangerous Goods Packer in accordance with the relevant regulations);
- preparation of documents to be hand carried by a courier to the OPCW Laboratory (see below);
- providing information to the OPCW Laboratory for it to arrange for receipt of the samples and the preparation of control samples;
- arrangement of in-country transport of the sample to the Point of Exit by the ISP;

Samples are packed in OPCW-approved sample transport containers (see Section 2.1.3) that fulfil the requirements of IATA regulations and ICAO (International Civil Aviation Organization) Packing Instruction 623 for air transport ⁽⁶⁾. Each transport container is used only for the splits of one authentic sample; background/blank samples will not be sent off-site. The 4–5 sealed sample vials of the same authentic sample are weighted and placed into a secondary container(s); its void space is filled with absorbent material. Each secondary container is sealed with OPCW frangible seals. Secondary containers are packed into one intermediate stainless steel packaging that is capable of withstanding a free drop, penetration, and heat. The intermediate packaging is sealed with OPCW fibre optical seals. One set of photographs of the optical end pattern of the seals is handed to the ISP, additional sets are hand carried by an OPCW escort to the Technical Secretariat. If so requested, the ISP may place, in addition,

its own seal on the intermediate packaging. The intermediate packaging is placed into a transport over-pack container (wood or stainless steel) for shelter during transport. The over-pack container is marked with a unique tag to be easily identified, secured with wire or plastic tags against unintended opening, and marked with the proper dangerous goods labels.

Samples are shipped under UN number 3315 'Chemical sample, toxic, liquid or solid' (class 6.1). These samples are forbidden to be transported by air except under Special Provision A 106, which allows for samples to be shipped in connection with the implementation of the Chemical Weapons Convention ⁽⁶⁾. Under this provision, these samples can be transported on a passenger or cargo plane providing prior approval is granted by the appropriate authority of the state of origin or the Director-General of the OPCW. However, final acceptance of the dangerous goods shipment under Special Provision A106 is at the discretion of the operator (airline pilot). Because samples will always be transported by road as well (i.e. from the airport to the OPCW Laboratory) the shipment must follow appropriate regulations concerning the international carriage of dangerous goods by road, such as ADR (Accord Européen Relatif en Transport International des Marchandises Dangereuses par Route).

Transport and delivery of sample containers from the Point of Exit to the OPCW Laboratory and from the OPCW Laboratory to the designated laboratories is subcontracted to a specialist company. The Director-General will appoint an OPCW staff member to escort the samples during transportation to the extent possible and to hand carry the respective documentation.

On their arrival at the OPCW Laboratory, all seal numbers and the end pattern of the fiber-optic seals will be compared to the seal numbers and photographs received from the inspection site, if so requested in the presence of an ISP representative. Unpacking will be performed in a dedicated area for handling of authentic samples in the OPCW Laboratory. The vials containing the authentic sample splits will be weighted but left closed and sealed as received from the inspection site. For dispatch to each designated laboratory, one authentic sample split is packed together with a pre-analyzed control sample and the respective matrix blank in a transport container in the same manner as described above. A

designated laboratory receives a set of three nonindicated, sealed vials containing the authentic sample, the control sample, and the matrix blank. The vials and the respective accompanying documents provide no information in regard to their origin or the contents.

A schematic overview of activities related to sampling and analysis on-site and off-site is given in Figure 3:

4 THE CHAIN OF CUSTODY AND CONFIDENTIALITY OF INFORMATION

Chain-of-custody procedures are followed to guarantee the integrity and confidentiality of sample material during its lifetime from the collection of the sample up to its destruction.

A sample is under OPCW custody, if

- it is in the physical possession of an OPCW staff member;
- it is in the view of an OPCW staff member after having been in his/her physical possession;
- it has been in the physical possession of an OPCW staff member and placed by him/her in a designated and identified secured area sealed with an OPCW tamper proof seal.

During transport, OPCW custody of samples is maintained by the OPCW seals (fibre optical, fragile) on the primary, secondary, and intermediate packaging containers. The integrity of these seals ensures the integrity of the samples in regard to tampering.

Movements of samples are performed in a controlled and documented manner, which will enable the life history of each sample or portion thereof to be tracked. This is achieved by means of appropriate documentation (see below) and the presence of an OPCW representative to escort samples to the extent possible during transport. The information contained on the shipping documentation must be limited to the minimum information necessary for the transfer of samples and as required by the respective transport regulations.

There are three types of material containing information on a sample:

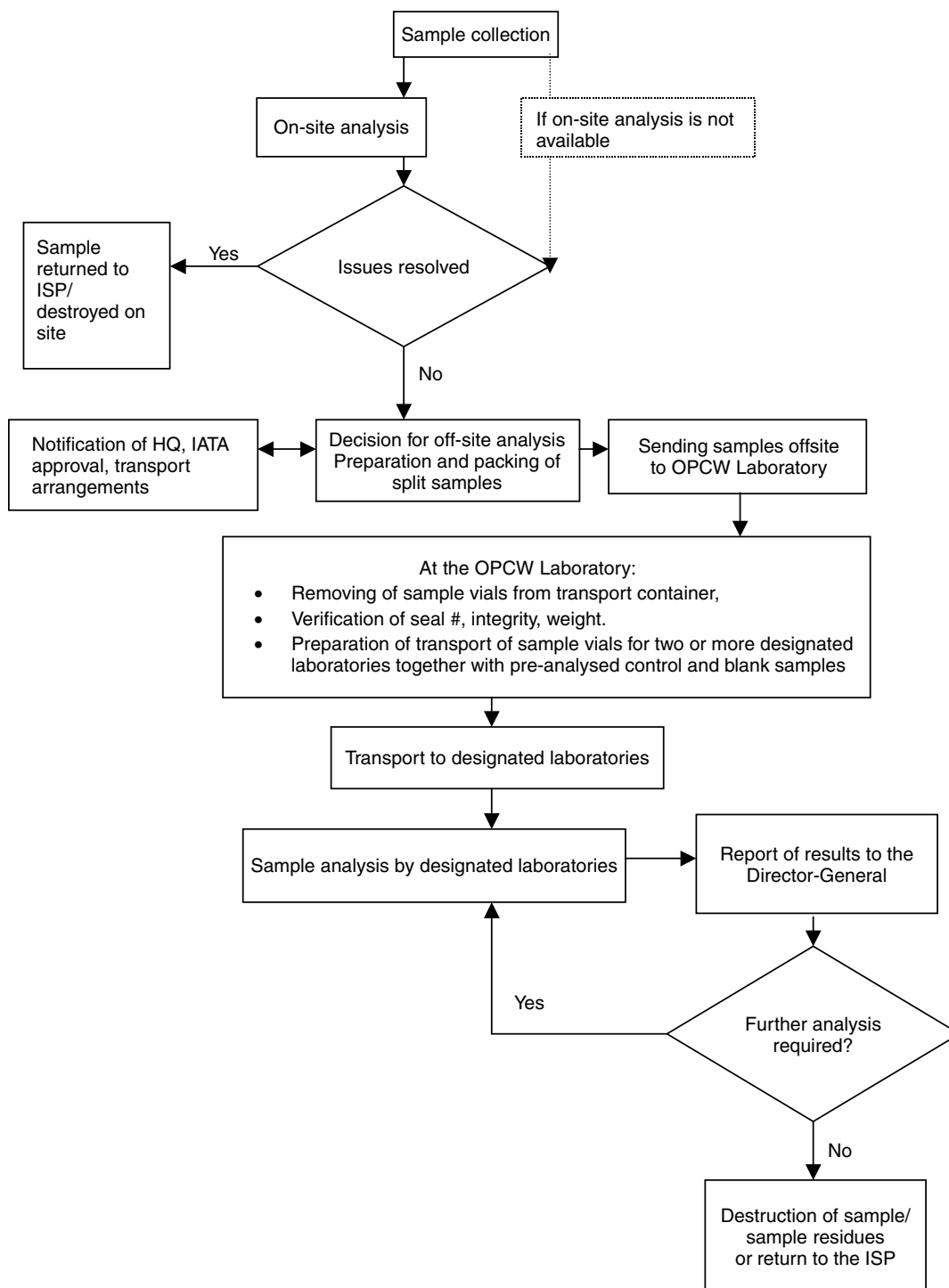


Figure 3. Scheme of sampling and analysis activities

- the sample itself;
- the hard disk of the GC/MS computer holding the sample analysis information;
- documents concerning sample collection, preparation, and analysis.

The authentic samples represent classified material different to the material generally collected or created during an inspection. The sample contained in a vial offers very little information in itself. However, it becomes a powerful source of information combined with the details about its origin and its analytical results. Therefore, the sample and any information on its origin or content must be separated.

The hard disk of the GC/MS computer is purged on site after sample analysis was concluded in accordance with OPCW procedures. It may be retained by the ISP, if so requested.

5 DOCUMENTS

The following documents are related to sampling and analyses during on-site inspection activities:

- At the beginning of an inspection, an individual *inspection notebook* is issued to each IT member. The inspector must record all information gathered during the inspection preparation phase and on-site activities in his/her inspection notebook. The notebooks never leave the custody of the IT.
- All activities and conditions regarding sampling, sample splitting, sample preparation, analysis, and destruction of sample material on-site are documented in the *OPCW Sampling and Analysis Booklet*. One booklet is used for each authentic or background sample collected on site. Sample code of the authentic sample and seal numbers of the authentic and the split samples are recorded in this booklet. Each page filled in the booklet is authenticated by the inspector(s) performing the task and offered to the ISP representative witnessing the activity to confirm it with his/her signature. The booklet is closed when all on-site activities concerning the respective sample are finalized. The booklet never leaves the custody of the IT; it is part of

the on-site analysis report (see below) and as such hand carried from the inspection site to the OPCW Technical Secretariat.

- All documents related to and created during on-site sampling, sample splitting, sample preparation, and analysis are attached to the *on-site analysis report* (see below). The on-site analysis report is part of the report created by the IT concerning the inspection activities and subject to the same confidentiality regime as the report itself. Under no circumstances are any of these documents sent together with samples off-site. The on-site analysis report has to provide data to validate the method as well as the analysis result in accordance with OPCW procedures and the Quality System requirements.
- OPCW internal forms for handover of confidential material and erasure of computer hard disks. The documents do not contain any confidential information themselves but record transfers and destruction of confidential material.

6 REPORTING

All activities and factual findings during the inspection period are reported in the Preliminary Findings (PF, for all inspections except CWDF) or monthly Interim Report (IR, for continuous monitoring CWDF inspections) (see CWC VA Part II Paragraph 60). An inspection report includes annexes concerning samples taken and analytical results. As part of these Annexes, an on-site Analytical Report is attached, which consists of

- the OPCW Sampling and Analysis Booklet for each sample (see above);
- all documents related to GC/MS analysis including QA/QC (quality assurance/quality control) measures, tuning reports and performance check and calibration runs (see above);
- the on-site analysis report, a compilation of personnel equipment, chemicals involved, and procedures used during on-site S&A activities as well as observations and comments concerning the analytical results.

The ITL and a representative of the ISP sign the inspection report, a copy is handed to the ISP, and the report is hand carried by the IT to the OPCW Headquarters.

7 PACKING OF EQUIPMENT AND RETURN TO HQ

When on-site activities in regard to sampling and analysis are completed, the OPCW equipment will be cleaned and – if necessary and possible – decontaminated. Reusable items that cannot be decontaminated, all disposable items that have been removed from their original packing, and all original packing and chemicals that have been opened are left on site. Parts of the equipment that may contain information unrelated to the purpose of the inspection that cannot be removed may be taken under joint seal to the OPCW headquarters or left on site, if so requested by the ISP (e.g. parts of the GC/MS system, the GC column, the injector liner, the purged hard disk) ⁽⁷⁾. Remaining portions of the samples and unused split samples are returned to the ISP. Prepared samples can be destroyed in accordance with OPCW procedures ⁽¹⁾ or handed over to the ISP. IT members packing the equipment will complete detailed packing lists for equipment returned to the OPCW equipment store. After cleaning, the equipment is packed into its transport boxes. The ISP has the right to check the equipment during Point of Exit procedures; the transport boxes are then sealed with OPCW seals.

All waste created during sampling and analysis on site will be collected by the inspection team, decontaminated if necessary, labelled according to site specific waste disposal requirements, and handed to the ISP.

The equipment will be brought by the IT or by cargo to the OPCW equipment store. The contents of the transport cases will be checked by the equipment store personnel in the presence of an IT member. The IT will provide detailed reports about equipment left on site as well as malfunction or damage of equipment, if applicable. Necessary repair, maintenance, or calibration of equipment is performed or initiated by the OPCW Technical Support Branch.

ACKNOWLEDGMENT

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My thanks and acknowledgements to all my OPCW Analytical Chemist colleagues who drafted, improved, and revised procedures related to on-site S&A activities and to their dedication and effort to work under unusual and sometimes difficult conditions.

ABBREVIATIONS AND ACRONYMS

ADR	Accord Européen Relatif en Transport International des Marchandises Dangereuses par Route
AMDIS	Automated Mass Spectral Deconvolution and Identification System
BSTFA	<i>N,O</i> -bis-(Trimethylsilyl)trifluoroacetamide
CI	Challenge Inspection
CW	Chemical Weapons
CWC	Chemical Weapons Convention
CWDF	Chemical Weapons Destruction Facility
CWSF	Chemical Weapons Storage Facilities
DMT	Dimercaptotoluene
GC/MS	Gas Chromatograph/Mass Spectrometer
HCB	Hexachlorobenzene
HPLC	High Performance Liquid Chromatography
IATA	International Air Transport Association
IAU	Investigation of Alleged Use
ICAO	International Civil Aviation Organization
IPE	Individual Protective Equipment
IR	Interim Report
ISP	Inspected State Party
IT	Inspection Team
ITL	Inspection Team Leader
OIO	Office of Internal Oversight
PF	Preliminary Findings

POE	Point of Entry
QA/QC	Quality Assurance/Quality Control
S&A	Sampling and Analysis
SCBA	Self Containing Breathing Apparatus
SPE	Solid Phase Extraction
THF	Tetrahydrofurane
VA	Verification Annex

QDOC/LAB/WI/ SP004	Preparation of Soil Samples On-site for GC/MS Analysis
QDOC/LAB/WI/ SP005	Preparation of Wipe and Non-soil Solid Samples On-site for GC/MS Analysis
QDOC/LAB/WI/ SP006	Preparation of Organic Chemical (Solid or Liquid) Samples On-site for GC/MS Analysis

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QDOC/LAB/WI/ GCMS002	Bruker EM 640S GC/MS Testing and Preparation of Instruments for On-site Analysis
QDOC/LAB/WI/ GCMS003	Bruker EM 640S Portable GC/MS Packing Procedures
QDOC/LAB/WI/ GCMS004	Bruker EM 640S Portable GC/MS Installation and Handling of Software
QDOC/LAB/WI/ SC001	Collecting and Splitting Samples under Toxic and Hazardous Conditions On-site
QDOC/LAB/WI/ SC002	Splitting of Samples On-site
QDOC/LAB/WI/ SC003	Collection of Solid Samples On-site
QDOC/LAB/WI/ SC004	Collection of Liquid Samples On-site
QDOC/LAB/WI/ SC005	Collection of Wipe Samples On-site
QDOC/LAB/WI/ SP003	Preparation of Aqueous Samples On-site for GC/MS Analysis

- | | |
|-----------------------|--|
| QDOC/LAB/WI/
SP008 | Chain of Custody and
Documentation During the
Inspection Period |
| QDOC/LAB/WI/
SP009 | Handling of Authentic Samples
from Inspection Sites at the
OPCW Laboratory |
| QDOC/LAB/WI/
SPO10 | Packing and Transport of
Off-Site Samples |
| QDOC/LAB/WI/
SP016 | Destruction of Sample Material |
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CHAPTER 4

The OPCW Gas Chromatograph/Mass Spectrometer for On-site Analysis. Instrumentation, AMDIS Software and Preparations for Use

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1 INTRODUCTION

Sampling and analysis (S&A) during inspections is one of the verification tools provided for by the Chemical Weapons Convention (CWC) ⁽¹⁾. Chemical analysis of a sample is the only direct and scientific (and not only circumstantial) tool to confirm the presence of a chemical substance. The presence of a declared chemical in a declared place at a declared time and in declared quantities confirms the declaration provided by a State Party to the OPCW (Organization for the Prohibition of Chemical Weapons). The actual or past presence of a chemical, which should not be at the inspected site according to the declarations, or, which has

been used for purposes prohibited under the CWC is a very important observation in the verification process. This is especially true in the cases when a chemical was present in the past but was removed (degraded) due to natural factors or intentional action. Finding characteristic degradation products may provide important information for verification.

The compounds listed in the three Schedules of Chemicals in the CWC differ very much in their physical–chemical properties. They need to be detected and identified as bulk substances in high concentrations as well as at ppm levels in environmental samples. This demands sophisticated methods of sample preparation and instrumental analysis. From a practical point of view, this

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can only be achieved by GC/MS (gas chromatography/mass spectrometry) analysis and thus, GC/MS instruments and analysis were approved by OPCW to be used for on-site analysis ⁽²⁾.

Traditional GC/MS analysis and data processing could reveal the identity of chemicals that are not related to the purpose of the inspection. Under certain circumstances this may not be acceptable for the inspected State Party (ISP) because the information may be considered as confidential for business or for national security reasons.

Therefore, the information obtained, through on-site GC/MS analysis about the identity of compounds present in a sample should be restricted to chemicals relevant to the aim of the inspection. This is achieved by operating the instrument in a specially developed *blinded mode*, which shows neither the chromatogram nor mass spectra during or after the chromatographic run. Additionally, if the analysis is conducted in *blinded mode*, the only available postprocessing software is a specifically developed on-site version of AMDIS (Automated Mass Spectral Deconvolution and Identification System). This software works only with the OCAD, which contains only compounds relevant to the CWC; thus, it reports exclusively the presence of compounds for which spectra are in this database library.

2 INSTRUMENTATION

In 1996, an international team of experts evaluated the only two available portable GC/MS systems at this time: Viking SpectraTrak 672 (USA) and Bruker EM 640S (Germany). As the Viking SpectraTrak 672 instrument did not fulfill requirements concerning the possibility of being operated in *blinded mode*, only Bruker 640S passed successfully a series of tests, and was approved by OPCW for on-site analysis and became an item of approved inspection equipment.

2.1 Bruker EM 640S GC/MS System

The Bruker EM 640S is a transportable, modular GC/MS instrument consisting of a mass spectrometer, a gas chromatograph, a liquid sample

split/splitless injector, a gas supply, and an electronic peripheral module, an industrial ruggedized PC and an external power supply (see Figure 1).

The instrument can also be equipped with a desorber module, a dual desorber/split/splitless injector module or an automatic air sampler module. But these are not used in the current configuration of the system, which is approved for on-site analysis.

The mass spectrometer is equipped with an EI ion source, operating in 70 eV/200 μ A standard condition. The ion source has two filaments that can be switched by the software. An all-glass, one-piece quadrupole filter is used as mass analyzer. The detection system consists of two channel electron multiplier detectors, one operating in counting, the second in analog mode. The ion beam leaving the quadrupole filter is electronically switched between the two multipliers based on the intensity of detected ion signals, thus extending the linearity range. The mass range is 2–640 amu with a variable peak width 0.1–4 amu (standard settings: unity resolution with 10 % valley definition). Maximum scan speed is 2000 amu/s.

The vacuum system of the instrument consists of a four-stage, internally integrated membrane pump with an ultimate pressure of about 1–2 mbar. The final vacuum is reached with a 70 L/s turbo molecular pump.

The GC module used by the EM 640S is a specially designed miniature GC oven containing a standard GC capillary column. The column is coiled with one layer of 68 mm diameter between

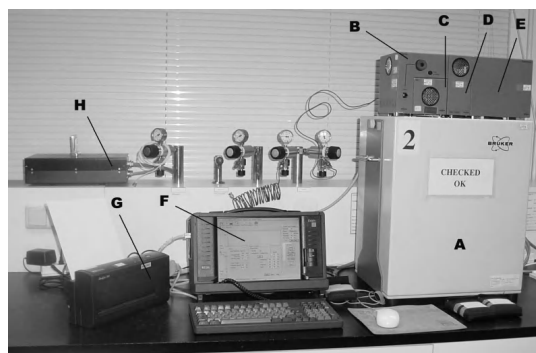


Figure 1. Bruker EM 640S modular GC/MS system: A – mass spectrometer, B – split/splitless injector, C – GC module, D – gas supply, E – electronic module, F – ruggedized PC, G – Printer, H – external power supply

two layers of thin polyimide foil on an external wall of a cylindrical, low heat-inertia column heater. The outlet of the column enters directly into the ion source of the mass spectrometer through a specifically designed, temperature-controlled inlet system. The inlet system is sealed by a silicon *O*-ring, which allows for a very quick change of the entire GC module in case of malfunction. On the inlet side of the column is a conical teflon™ connector that allows for quick connection to the injector module, which is mounted in the top of GC module (see Figure 1).

The HP5MS column used in OPCW Bruker 640S GC/MS systems is a carefully chosen brand of capillary column, which gives retention indices very similar to those contained in OCAD (OPCW Central Analytical Database).

The split/splitless injector module is a fully software-controlled system that is capable of performing the injection of the liquid sample in variable split, splitless, pressure pulse split, and pressure pulse splitless modes. The septum purge is controlled manually and is usually set for 2.5 mL/min. The temperature of the injector is software controllable up to 300°C. Vapors of the sample created in a silanized 2 mm internal diameter glass liner are transferred via a 0.53 mm id, 35 mm long deactivated capillary to a split block from where they enter the inlet of the column. The chromatographic process is performed by multiple ramp temperature programming with a maximum heating rate of 32°C/min up to 280°C. Flow and pressure control up to maximum of 1000 hPa allows for the choice between a constant pressure or constant flow chromatographic process. The operating parameters of the system are listed in Annex 1.

The external power supply for EM 640S requires power input of 110–240 V AC, 50–60 Hz, 1.5 kVA and has a power output of 24 V DC/1 kVA. The GC/MS system is operating on 24 V DC, maximum 600 VA.

A ruggedized PC is used for control of the mass spectrometer and the peripheral modules as well as for the acquisition, processing, storage, evaluation, and output of measured data. The Bruker ATLIF transputer interface card works as the interface to the mass spectrometer.

The PC is a DOLCH Intel Pentium 586 MMX, 233 MHz, 64 Mbytes RAM industrial computer with SVGA LCD color screen and mouse pointing device.

It has a 1.44 Mb floppy drive, CD reader and is operating from a single removable 2–5 Gbytes Disctec hard disk. Operating system is IBM OS2 Warp 3.0 with some Windows applications. The power requirement for the PC is 110–240 V AC, 50–60 Hz/5 A.

The presence of the only one and removable hard disk was requested by decision of Conference of the State Parties on its first session⁽³⁾. In Attachment 6 of the decision 71 concerning specific operational requirements for GC/MS system, it says that all data in nonvolatile memory must be retainable at the inspected site under joint custody of the Technical Secretariat (TS) and the inspected State Party. The hard disk with all raw data may be considered as containing highly protected information and requested to be left on site of inspection.

All parts of the system, which may contain information on the identity of the compounds present in analyzed samples may be left on site on request of ISP. It includes, as mentioned before, removable hard disk with GC/MS data but also injector septum and liner and other parts of the system, which came into contact with the sample.

If so agreed with ISP, GC/MS data on the hard disk may be wiped out by formatting the disk using a special programme provided with the system and the hard disk returns to Secretariat.

2.2 The Software

The GC/MS system works with BRUKER-m.a.c.s. LabStar, and BRUKER DataAnalysis software packages.

The BRUKER-m.a.c.s. LabStar control and data system controls the GC/MS measurement hardware and acquires and stores measured data. It works under an IBM OS2 Warp multitasking operating system. In addition to data acquisition and real time display of data currently acquired (mass spectrum and chromatogram), it also allows for data evaluated or processed by the BRUKER DataAnalysis software.

The BRUKER DataAnalysis software is a tool for processing and evaluating GC/MS data already acquired. It includes routines for deconvolution of overlapping spectra, generating of extracted ion chromatograms (EICs), integrating TIC (total ion chromatogram) and EIC signals, as well as

searching the mass spectral library. The default library searched by BRUKER DataAnalysis is the commercial NIST98 library containing more than 100.000 mass spectra.

In addition to the above mentioned software packages, which are standard for EM 640s, there are two other OPCW-specific, postprocessing data analysis programs: the open (laboratory) version of AMDIS and the on-site version of AMDIS. Both versions were developed by NIST and they are independent, powerful, very flexible GC/MS data analysis programs performing an automatic search of mass spectra of all chromatographic peaks against dedicated, user-built AMDIS libraries. The open version may, in addition, also make a search of the commercial NIST library, if the library and NIST Search98 are installed on the same computer system.

2.2.1 On-site Version of AMDIS

The on-site version of AMDIS is a 16-bit Windows application running under OS2 operating system of the Dolch PC. This version of AMDIS was specially designed by NIST (National Institutes of Standards and Technology) for OPCW on-site analysis use. Its role in the postprocessing of analysis data is equivalent to the role of the *blinded mode* software in regard to the general control of the instrument and the acquisition of the data: *restrict the information on the identity of chemicals present in the sample to chemicals that are only relevant to the inspection aim*.

The application of AMDIS is triggered automatically by the end of the chromatographic run. It works in the background and the results of intermediate processes are not visible on the screen. The program detects peaks in the process of deconvolution, isolates their spectra from the background and overlapping peaks and compares the 'pure' spectra of detected peaks with the spectra in the library currently used by AMDIS.

The library used by AMDIS depends on the type of data being analyzed. While triggering the on-site version of AMDIS, after the chromatographic run is completed, the operating system defines the type of run that has just been performed by passing the information on the chromatographic method used to AMDIS. There are two types of chromatographic

runs: *test runs* and *analytical runs*. Associated to those two types of chromatographic runs are two main and one auxiliary AMDIS libraries:

1. During processing of the *test run data* (injection of OPCW test mixture) AMDIS is searching a small, dedicated Chemical Standards Library (ASCII file onsite.csl). This library contains only data and spectra of the 16 compounds contained in the OPCW test mixture (see Annex 2, Table 1). A compound in the test mixture is defined as detected if the net match factor of the compound spectrum compared with library spectrum is ≥ 85 . This threshold of identification is fixed in the on-site version of AMDIS.
2. During processing of the analytical data from a sample (injection of a blank or an authentic sample) AMDIS is searching the dedicated on-site target library consisting of a Mass Spectra Library file and a Chemical ID file (ASCII files onsite.msl and onsite.cid). This library contains an extract of the mass spectra from the OPCW analytical database (see **Chapter 7**) approved for use in the verification process. The contents of the library actually transferred to the instrument hard disk are agreed between the inspection team and the ISP at the beginning of the on-site inspection activities. The library also includes the retention indices (RI) of most compounds. Retention index (RI) is a dimensionless parameter, which converts retention time of a given compound into the value that is less dependent on small changes in chromatographic conditions than retention time itself. Retention index is calculated on the basis of retention times of retention index standards in the same chromatographic system. OPCW procedure uses normal alkanes with even carbon number from C8 (octane) to C24 (tetracosane) as RI standards. OCAD contains several hundreds of RI of scheduled compounds that are included in on-site AMDIS library. Comparison of RI of detected compound with RI of the same compound present in the AMDIS library increases credibility of identification. Applying an 'RI penalty' may later lower the net match factor of the compound spectrum, for example, if the RI of a given chemical is not present in the target library or the RI of the

compound differs from the library value and if AMDIS is instructed to do so by software settings. OPCW on-site settings of the software do not apply an 'RI penalty', thus giving the operator the opportunity to make an assessment of this issue. A compound in the sample is defined as detected if the resulting net match factor is ≥ 80 (this is the threshold for identification, which is fixed in AMDIS).

3. According to OPCW procedures, all GC/MS runs of a sample or a blank involve the co-injection of an internal standard (hexachlorobenzene contained in the OPCW HCB mixture). This is a quality control measure, which is applied to assess the validity of the run, especially when operating in *blinded mode*. When AMDIS processes data from the internal standard run, it uses a small auxiliary Internal Standard Library (ASCII file onsite.isl). This library contains data on HCB (mass spectrum, RI, etc.). HCB is defined as detected if the net match factor for HCB is ≥ 80 (this is the threshold for identification, which is fixed in this version of AMDIS). Everything so far is done by AMDIS in the background.

Finally, AMDIS displays on the PC screen its Result Window, which contains – *only for chemicals defined as detected* – the chemical name, retention time, RI, and some additional QA (Qualitative Analysis) parameters. The operator will consider the run as 'valid' only if HCB was detected with QA parameters fulfilling the acceptance criteria. There are other QA parameters that are reported by AMDIS after each run. They are peak width, peak tailing, solvent tailing, total ion background, and background for ion 207. Even in blinded mode when chromatogram is not visible, the values of these parameters allow operator to assess the quality of GC system, accept or reject the particular run, or take proper preventive action (bake or change the column or maintain injection system).

Further, the operator will evaluate individually each chemical detected by AMDIS to control if the QA parameters for this compound are fulfilled. The acceptance criteria are defined in OPCW quality procedures (see Annex 3).

The operator may, in addition to this, open the *Confirmation Screen* of AMDIS to evaluate the peak

shape of the detected compound and to compare the spectrum of this peak with the library spectrum. Finally, the operator makes a printout of the AMDIS report, which contains the list of detected compounds with their QA parameters. This printout also contains additional QA measurements of more general nature, which indicate the behavior of the instrument during this particular run. The amount of information available for detected compounds as well as special features connected with the operation of the on-site version of AMDIS, its security level filters, which allow further restriction of the amount of information displayed, are described in **Chapter 2**. The advantage of AMDIS was described already in Section 2.2. AMDIS performs automatic analysis of chromatograms containing sometimes hundreds of peaks. Limitations of AMDIS are caused, mainly, by character of target on-site library, which contains spectra confined to scheduled chemicals. It may lead to false negative (lack of identification of compound present in the sample) if the spectrum is not present in the library (e.g. riot control agent). False positive occurs when AMDIS misidentified compound because its spectrum is very similar to the spectrum of another compound present in the library. The example of similarity spectra, which may lead to false positive, is presented in Figure 2. However, the value of RI of unknown compounds reported by AMDIS will solve ambiguity. These two compounds have very similar spectra but significantly different retention indices.

2.2.2 Open Version of AMDIS

The open version of AMDIS is only available when the instrument is operated in *open mode*. In addition to the functions of the on-site version of AMDIS as described above, the open version allows for displaying the entire chromatogram and the spectra of all peaks present in it. Chromatographic peaks and their spectra are shown independently from any match of the spectrum to the mass spectra present in the library used. The open version of AMDIS is not restricted to the use of the on-site target library onsite.msl but can utilize any other AMDIS library installed.

The operator has unlimited flexibility in changing different settings of AMDIS including the net

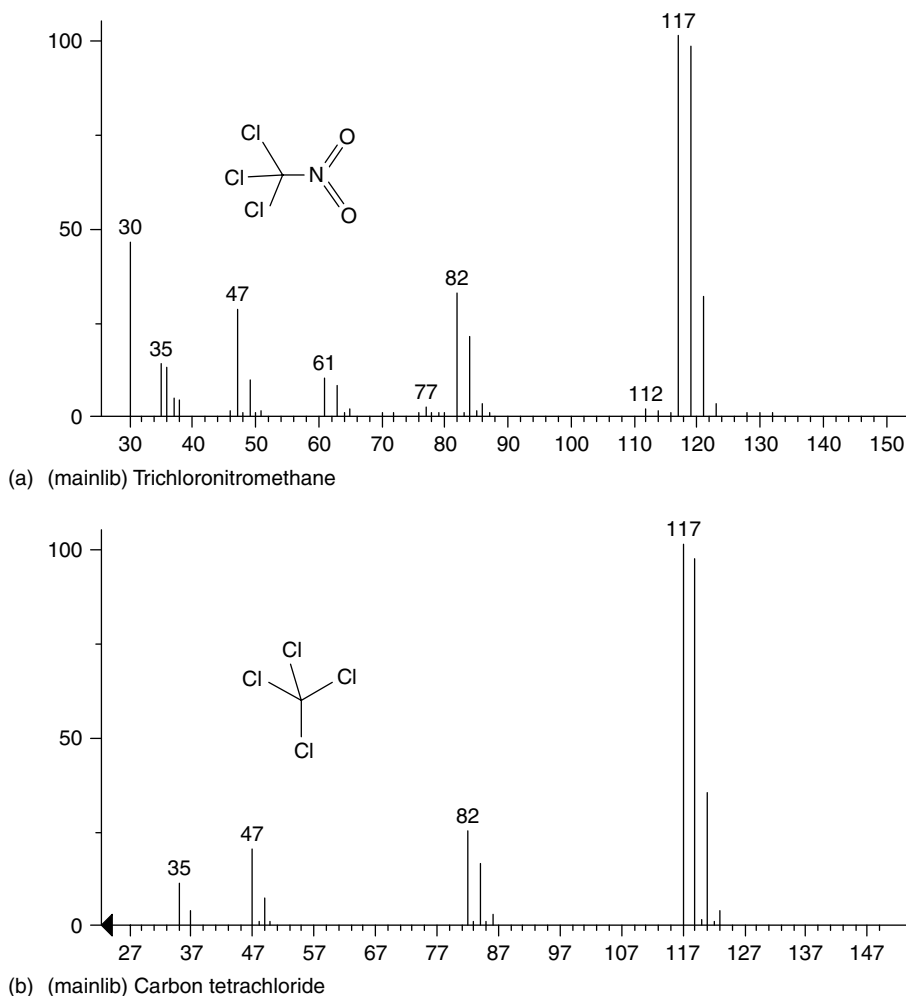


Figure 2. Similarity of mass spectra of (a) scheduled compound chloropicrine and (b) nonscheduled compound carbon tetrachloride

match factor threshold for identification, which is fixed in the on-site version of AMDIS. The open version allows for browsing of data in libraries as well as managing the content of libraries (editing and transferring the data between libraries) and building a user library using data from processed GC/MS runs.

The restrictions that can be applied by selecting the security level filters of AMDIS in the negotiation module (*see Chapter 2*), which influence the output of data from the on-site version of AMDIS as explained above, do not apply for the open version.

3 MANAGING OF INSTRUMENTS AT THE OPCW LABORATORY

The OPCW owns five Bruker EM 640S GC/MS systems for performing on-site analysis during inspections. The OPCW Laboratory – which is a part of Technical Support Branch of the OPCW Technical Secretariat – is responsible for the management of the systems. This implies:

- keeping the systems in working order;
- preparation and validation of instruments to be sent for a mission;

Table 1. Procedures for testing a GC/MS system before being packed and sent for a mission

QDOC/LAB/WI/GCMS002	Maintenance, set-up, testing of hardware and software components
QDOC/LAB/WI/GCMS003	Packing
QDOC/LAB/WI/GCMS004	Installation of software components

- checking of the status of each instrument after returning from the mission;
- maintenance (such as ion source cleaning, replacement of septa, liners, etc);
- arranging of regular and troubleshooting maintenance by the supplier.

Before a GC/MS system is packed and sent for a mission, it is tested according to the procedures that are contained in the respective work instructions listed below (Table 1). After completion of the testing, the OPCW Office of Internal Oversight (OIO) audits if all criteria have been met. If satisfied, this office issues a certificate for the system, which is valid for the use of the system for a particular mission. The OPCW Laboratory is accredited for this process by the Dutch Accreditation Council (RvA) in accordance with the requirements of ISO/IEC 17025.

3.1 Principles of Testing and Preparing the Instrument

The procedures described in the work instructions will assure that

- (a) GC/MS instruments are prepared and kept ready for inspections in a waiting mode in the OPCW Laboratory;
- (b) the appropriate validation reports are provided only for instruments that meet the acceptance criteria; and
- (c) instruments returned to the OPCW Laboratory are checked and maintained.

Different sequences of procedures are applied on an instrument:

- (a) during testing and preparation for use on a mission, training or exercise; *Exit Control*

- (b) while waiting in the OPCW Laboratory: *Waiting Status Control*;
- (c) returned from use on a mission, training or exercise or from Bruker maintenance: *Entrance Control*.

Each GC/MS instrument is maintained in the Quality-Control/Service/Packing circle as shown in the flowchart in Figure 3.

The process of preparation of a GC/MS instrument for use during inspections or training outside the OPCW Laboratory as well as for in-house exercises, which requires certification by the OIO involves:

- (a) set-up of hardware components;
- (b) installation of software components;
- (c) testing of hardware and software components;
- (d) maintenance;
- (e) packing; and
- (f) certification of testing and packing by OIO.

3.2 Installation of the Software

For every instrument to be sent on an inspection, two identical hard disks containing the system software are prepared using an installation source CD provided by Bruker Bremen. The installation CD creates an image of the software package on the hard disk. Bruker Bremen developed this in cooperation with NIST and the OPCW Laboratory. The software is periodically updated. The version in use is 3.a4q from 2000. The system software package consists of LabStar version V-0440.5 PVSC 1.24, Bruker Data Analysis version 1.0i and AMDIS open and on-site version 1.0. Each installation of the software is given a unique identification number, which is visible in the Installation Specifier (see Figure 4). This unique identification number is also the name of the directory containing all GC/MS raw data.

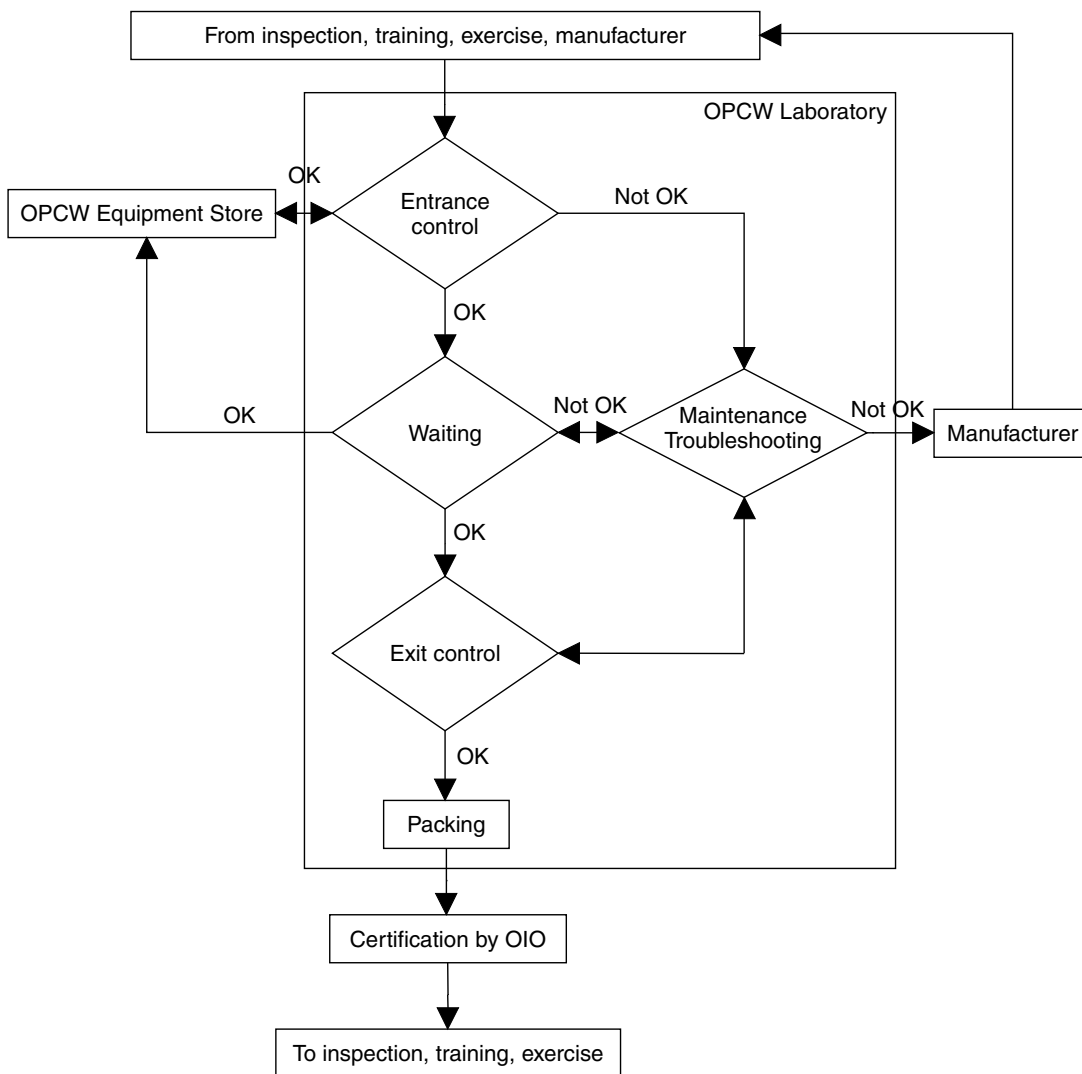


Figure 3. QA/QC flow diagram of GC/MS maintenance

The installed software package also contains the AMDIS libraries onsite.cls and onsite.isl for calibration, test, and internal standard compounds (see Section 2.2). After software installation the hard disks are assigned to a particular GC/MS system. Since each instrument (defined by serial number) may be combined with any of the 5 electronic modules, 12 GC modules and 10 injector modules in possession of OPCW the actual instrument configuration needs to be defined. This is achieved by installing configuration files for the

hardware components being actually used with that system (1 electronic module, 2 injectors, 2 GCs, see Section 3.3) on the two hard disks with a tool called 'Installation Specifier' (see Figure 4).

Configuration files for hardware components are saved on the hard disks in directories in which names indicate the serial number of the particular component, for example, files for injector serial number 34 are located in the directory SI00034, files for column over serial number 7 are located in the directory 2G00007. The recent tuning parameters for

Figure 4. An example of Installation Specifier for GC/MS no.6 equipped with GC columns no. 23 and 47 and Injector modules no. 6 and 16

the ion source and the multipliers of given system are stored in the directory of the mass spectrometer (i.e. ET00006 for mass spectrometer number 6) and updated according to the actual settings of a particular instrument.

3.3 Operational Parameters and Predefined Methods

Each GC/MS system is tested and configured for an inspection with two injectors and two GC modules/columns. These four modules allow creating four different instrument configurations, which need to be installed on the particular hard disk in order for the software to be able to control them. One

instrument configuration is defined by combination, for example, 'MS 6 + column 23 + injector 6' or 'MS 6 + column 47 + injector 16', and so on (see Figure 4).

The instrument software is set to offer eight predefined analytical methods. All common operational parameters for these methods are presented in Annex 1. The eight analytical methods are as follows:

1. Preparation (injector/ES)
2. Performance check injection
3. Solvent blank injection
4. Solvent extract analysis
5. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) blank injection

6. BSTFA analysis
7. Dimercaptotoluene (DMT) blank injection
8. DMT analysis.

Each method is performed under constant flow conditions and uses the same temperature/carrier gas pressure program (see Annex 1). This ensures that the retention times of analytes are unchanged and the RI calibration table used for the calculation of RI by AMDIS is valid for each run. The solvent delay times vary between methods to adjust for the type of solvent used for a particular injection. The preparation run is different: the temperature program heats faster and no data are acquired. This run is used only for the preparation and/or baking/cleaning of the system.

3.4 Testing and Validation of the System

Two instrument configurations of the four possible for a GC/MS system (Inj.1 + GC1 and Inj.2 + GC2) are tested and validated by performing a series of chromatographic runs and evaluating their results. Both hard disks and both instrument configurations

must be tested successfully in order for the system to obtain 'OK status'.

The performance of the system is tested by injecting 2 μ l of OPCW check mixture running the 'Performance Check Injection' method. The composition of the mixture is given in Annex 2. The retention times of the series of nine hydrocarbons in the check mixture are used by AMDIS for calibration of the RI; the other seven compounds are used for assessing the performance of the GC and MS part of the system. Two components of the check mixture, chloromethylaniline and dibenzothiophene, are used for evaluation of isotopic ratios for chlorine and sulfur measured by the mass spectrometer. The example chromatogram of the check mixture is presented in Figure 5.

The data of three consecutive injections of the check mixture are processed by AMDIS and evaluated by the operator. The results of testing are recorded in a check list and if they fulfill the acceptance criteria given in Annex 3 each system component is labelled separately 'OK' when in 'waiting status' in the OPCW Laboratory or 'Accepted for on-site use' when tested for exit control (see Figure 3). The waiting status evaluation

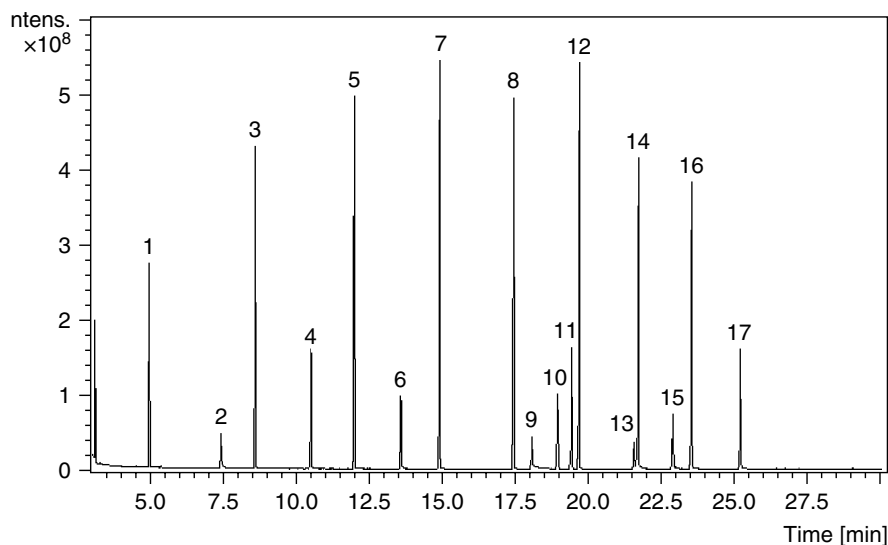


Figure 5. An example of TIC of the check mixture from testing of 640S: 1 – octane, 2 – Trimethylphosphate, 3 – decane, 4 – dimethylphenol, 5 – dodecane, 6 – chloromethylaniline, 7 – tetradecane, 8 – hexadecane, 9 – tributylphosphate, 10 – HCB, 11 – dibenzothiophene, 12 – octadecane, 13 – malathion, 14 – eicosane, 15 – methyl stearate, 16 – docosane, 17 – tetracosane

'OK' is valid for three weeks, after that testing is repeated.

If a system was returned from inspection, external exercise, or maintenance at the manufacturer, an additional test for cleanliness (entrance control) is performed. Three different blank injections with solvent, BSTFA, and DMT are performed and evaluated using an on-site target library containing all MS spectra from the OCAD. Absence of any identification ensures that the system components are free from scheduled chemicals and their degradation products. The same blank injection tests are performed if a system in waiting status was used internally for injections other than check mixture. The on-site target library used in the test of cleanliness is removed from the hard disk before issuing the system for a mission.

3.5 Packing of the System

A GC/MS system with a valid 'OK status' (not older than two weeks) can be issued by the OPCW Equipment Store after a final exit control (see Figure 3). The exit control consists of two test injections, one for each system configuration, of OPCW test mixture from the same batch as issued for the inspection. The exit control is performed in the presence of an analytical chemist inspector that will operate the system during the inspection. The same inspector is also participating in the process of packing of the system. A checklist for packing is followed step by step. The instrument, its different modules, auxiliary equipment tools, and spare parts are packed in five custom-made flight cases (packing list, see Annex 4). The items below are packed and issued together with the system.

1. A Helium connection kit consisting of necessary preprepared tubing, regulators, tools and 2L/200 atm bottle of high purity helium. With the highest consumption of 50 ml He/min, this will allow for several weeks of independent operation of instrument.
2. The commercial NIST MS library on CD.
3. Two certified floppy disks with copies of on-site OPCW Central Analytical Database (OCAD) are issued.

ABBREVIATIONS AND ACRONYMS

AMDIS	Automated Mass Spectral Deconvolution and Identification System
BSTFA	<i>N,O</i> -bis(Trimethylsilyl) Trifluoroacetamide
CWC	Chemical Weapons Convention
DMT	Dimercaptotoluene
EICs	Extracted Ion Chromatograms
GC/MS	Gas Chromatography/Mass Spectrometry
HCB	Hexachlorobenzene
ISL	Internal Standard Library
ISP	Inspected State Party
MSL	Mass Spectra Library
NIST	National Institutes of Standards and Technology
OCAD	OPCW Central Analytical Database
OIO	Office of Internal Oversight
OPCW	Organization for the Prohibition of Chemical Weapons
QA	Qualitative Analysis
RI	Retention Index
RvA	Dutch Accreditation Council
S&A	Sampling and Analysis
TIC	Total Ion Chromatogram
TS	Technical Secretariat

REFERENCES

1. *Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction*, Signed in 1993, Printed and Distributed by OPCW.
2. *Standard Operating Procedure (SOP) SOP-LAB-EQP-005, Bruker EM640S Portable GC/MS with AMDIS Data Analysis Software (Blinded Mode) for Qualitative Analyses of Liquid Samples Prepared by On-site Methods*, Version 3, The Technical Secretariat of the Organization for the Prohibition of Chemical Weapons, The Hague, 1999.
3. C-I/DEC.71, Attachment 6, *First Conference of State Parties*, The Hague, 1997.

ANNEX 1

Bruker EM 640S GC/MS parameters

Column: HP5MS, 30 m, 0.25 mm, 0.25 μ m

Injector: 250 °C

Connector: 150 °C

Interface: 150 °C

Inlet: 230 °C

Split mode: splitless

Splitless time: 45 s

Injector flow:

(i) general: 50 ml/min

(ii) during injection: 3 ml/min

Column pressure during injection:

300 hPa

Special standby:

(i) Column head pressure: 500 hPa

(ii) Injector flow: 10 ml/min

Temperature/pressure program: at 40 °C for 2 min at 500 hPa, increase from 40 °C to 280 °C at a rate of 10 °C/min; at 280 °C for 10 min

Total analysis time: 36 min

Carrier gas: helium, temperature compensated flow

Mass range: 40–450 amu

(a) Acquisition time: per mass
2.048 ms; total scan 841.7 ms

Solvent delay time and acquisition start depends on the method and are as follows:

Method	Solvent delay [min]	Acquisition start [min]
Preparation (Inj/ES)	9	8
Performance Check Injection	2.4	3.0
Solvent Blank Injection	2.4	3.0
Solvent Extract Analysis	2.4	3.0
BSTFA Blank Injection	7.4	8.0
BSTFA Analysis	7.4	8.0
DMT Blank Injection	14.9	15.5
DMT Analysis	14.9	15.5

ANNEX 2

Table 1. Chemical composition of the OPCW GC/MS test mixture

Chemical name	Abbreviation	Molecular formula	CAS Register Number
Trimethyl phosphate	TMP	C ₃ H ₉ O ₄ P	512-56-1
2,6-Dimethylphenol	DMPH	C ₈ H ₁₀ O	576-26-1
5-Chloro-2-methylaniline	5Anil	C ₇ H ₈ ClN	95-79-4
Tri- <i>n</i> -butyl phosphate	TBP	C ₁₂ H ₂₇ O ₄ P	126-73-8
Hexachlorobenzene	HCB	C ₆ Cl ₆	118-74-1
Dibenzothiophene	DBTh	C ₁₂ H ₈ S	132-65-0
Malathion	Mal	C ₁₀ H ₁₉ O ₆ PS ₂	121-75-5
Methyl stearate	MeSt	C ₁₉ H ₃₈ O ₂	112-61-8
<i>n</i> -Octane	c8	C ₈ H ₁₈	111-65-9
<i>n</i> -Decane	c10	C ₁₀ H ₂₂	124-18-5
<i>n</i> -Dodecane	c12	C ₁₂ H ₂₆	112-40-3
<i>n</i> -Tetradecane	c14	C ₁₄ H ₃₀	629-59-4
<i>n</i> -Hexadecane	c16	C ₁₆ H ₃₄	544-76-3
<i>n</i> -Octadecane	c18	C ₁₈ H ₃₈	593-45-3
<i>n</i> -Eicosane	c20	C ₂₀ H ₄₂	112-95-8
<i>n</i> -Docosane	c22	C ₂₂ H ₄₆	629-97-0
<i>n</i> -Tetracosane	c24	C ₂₄ H ₅₀	643-31-1

ANNEX 3

1. Acceptance criteria for Quality System parameters reported by AMDIS

1.1 The required results of the retention time calibration and performance test are as follows:

- (a) Not less than 15 calibration compounds are reported (HCB is not reported as a calibration compound).
- (b) The retention indices of not less than 5 of the 7 test compounds in the mixture differ from the expected values by no more than ± 5 units, the RI of the other do not differ by more than ± 10 units.

Each of the following criteria (e-g) should be met by at least 12 of the 16 compounds, the deviation of the other compounds should not be greater than by a factor 2 of the given value:

- (c) The retention times of the compounds in the mixture differ from the expected values by no more than ± 0.5 min. The retention times for the last five compounds may differ ± 1 min from the expected values.
- (d) The library search matches are higher than 85 (net). Note: Peaks with a high intensity may not meet this criteria due to the instrument specific detector, for these peaks a lower match factor can be accepted.
- (e) The peak width of the compounds is no more than three scans, except for

trimethyl phosphate, whose peak width is no more than four scans.

- (f) The peak tailing factor of the compounds is greater than 0.5 but not greater than 3, except for trimethyl phosphate, whose peak tailing factor is not greater than 4.
- (g) The signal to noise ratio (S/N) of the compounds is higher than 500, except for trimethyl phosphate and tributyl phosphate, whose S/N is higher than 300.

The following criteria for solvent tailing, background level and bleeding must be fulfilled:

- (h) The solvent tailing indicator reaches the values $S/N = 100$ within 3.5 min and $S/N = 50$ within 6 min, after beginning of the analysis.
- (i) The background level indicator reaches a ratio of median high to median low RT S/N ratio that is not higher than 10.
- (j) The bleed indicator reaches a ratio of median high to median low RT S/N ratio that is not higher than 10.

1.2 The isotopic ratios for the spectra of two compounds must meet the following criteria:

- 143/141 m/z ($^{37}\text{Cl}/^{35}\text{Cl}$) for 5-chloro-2-methylaniline is $(33 \pm 5.0) \%$
- 186/184 m/z ($^{34}\text{S}/^{32}\text{S}$) for dibenzothiophene is 3.4–8.4 %

ANNEX 4: EXAMPLE OF GC/MS PACKING LIST FOR INSPECTION**ORGANIZATION FOR THE PROHIBITION
OF CHEMICAL WEAPONS****VERIFICATION DIVISION
Technical Support Branch****Components of OPCW Inspection Equipment**

Equipment	Gas Chromatograph/Mass Spectrometer
Model, Type	EM 640 S
ID Number (Serial No.)	15309.0000#
Number of Boxes	5 (including Connection Kit)

Item No.	Box No.	Item	Seal No.*	Check Out	Left On-site	Check In
1.	A	Mass Spectrometer, Bruker EM 640 S				
2.	B	Split/splitless injector modules (Ser.#15317.000##, 15317.000##)				
3.	B	Electronics module (Ser.# 08101.000##)				
4.	B	GC separator modules (Ser.# 14017.000##, 14017.000##) -				
5.	B	Gas supply (Ser.# 11747.000##)				
6.	B	Spare parts				
7.	B	Toolkit				
8.	B	Nylon gloves				
9.	B	Snoop leak detector				
10.	B	Syringes, 10 µl Hamilton 1701N				
11.	B	Charcoal filter for outlet				
12.	B	Manuals				
13.	B	Validation reports (hardware/software)				
14.	B	On-site instrument logbook				
15.	B	Digital flowmeter				
16.	B	Membrane pump (spare)				
17.	C	External Power supply (Ser.# 14886.000##)				
18.	D	Computer, monitor, connection cables (Ser.# DCS 99011##)				
19.	D	DISCTEC removable hard disk with blinded software Ser # SSID #####				
20.	D	Printer (Ser.# #####)				
21.	D	NIST MS Spectra Library on CD				
22.	E	Helium Connection Kit (see separate list)				
23.		OPCW database disks (delivered separately)				

CHAPTER 5

Hazardous Environment Monitoring

George M. Murray and David S. Lawrence

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1 INTRODUCTION

When faced with an environment made hazardous by the presence of toxic chemicals, it is imperative to quickly identify the nature and extent of the hazard in order to take appropriate countermeasures. While the speed and accuracy of chemical analysis is steadily improving, devices suitable for field use are usually the result of a compromise. Thus, false-positive responses are prevalent due to the dire nature of a false-negative response. The only other option is to bring a full-scale laboratory to the site and there are military vehicles designed for this purpose. This option is impractical in most cases and so false-positive responses are currently the norm.

Spurred by recent events, there is an ever-growing interest in the detection of hazardous chemicals in both a military and a civilian context. The threat of chemical weapons has spread from the battlefield to cities and towns owing to the threat of international terrorism. Detection of hazardous chemicals and now chemical weapons is a requirement for first responders of all sorts. While a plethora of devices and materials exist, they all have certain inherent weaknesses and no one device can be relied upon to give an unambiguous response. As usual in chemical detection, two orthogonal methods give a much higher confidence than does any single method.

This report identifies options for sensing and responding to chemical agent releases. It

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

Edited by Markku Mesilaakso.

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comparatively evaluates current and near-term sensor options for detecting the most likely current threats. It also identifies sensor technologies that may be capable of responding to a wider range of substances (e.g. hazardous industrial chemicals, new chemical warfare agents) to provide options for coping with new threats as they develop. We collate previous research results on the principal background contaminants that have the potential for introducing unwanted false alarms in chemical agent detection systems, and suggest techniques that may be capable of reducing the impact of toxic material releases.

2 REAGENT-BASED TESTS

Historically, most first responders to toxic chemical releases have relied on reagent test kits to identify the chemical. When a liquid or gaseous agent is placed in contact with certain reagents, a change in color, fluorescence, or chemiluminescence may occur. Some reagents react with a variety of agents to give qualitative information while others simply give a 'yes' or 'no' response. These reagents are supplied in a variety of forms, such as papers, tubes, or vials. Some systems are semiautomated whereby the ambient air is drawn into a chamber in a continual flow by an electric air pump. The air then passes through an electrolyte medium and, if the target agent is present, it reacts in solution, generating

an electrical, colorimetric, or fluorescent signal to indicate its presence. Such automated systems are now becoming obsolescent in the defense arena as they become replaced by very effective technology that requires less maintenance, uses fewer reagents, generates fewer false alarms, and offers greater accuracy. For example, the UK-manufactured Nerve Agent Immobilized-enzyme Alarm and Detector (NAIAD) can detect any type of nerve agent (even currently unknown types) because it accurately mimics the natural process of acetylcholinesterase inhibition. It is also capable of detecting blood agents, such as hydrogen cyanide.

2.1 Detection Papers

The US Army issues M8 (Figure 1) and M9 detection papers. In the commercial sector, Anachemia Canada, Inc. (www.anachemia.com) provides the civilian equivalent detection in the form of Chemical Agent Liquid Detectors (C8, M-9, and 3-way). These papers are impregnated with agent-soluble pigments or dyes. Adhesive-backed papers from the M8 chemical detection booklets can be stuck on surfaces and clothing to provide warning of a liquid attack. The M8 paper gives immediate, qualitative verification of the presence of liquid V- and G-type nerve agents and H-type blister agents. In the case of the M8, yellow coloration on the paper signifies the presence of a nonpersistent nerve agent, red signifies the presence of a blister agent, while olive green or black signifies a persistent nerve

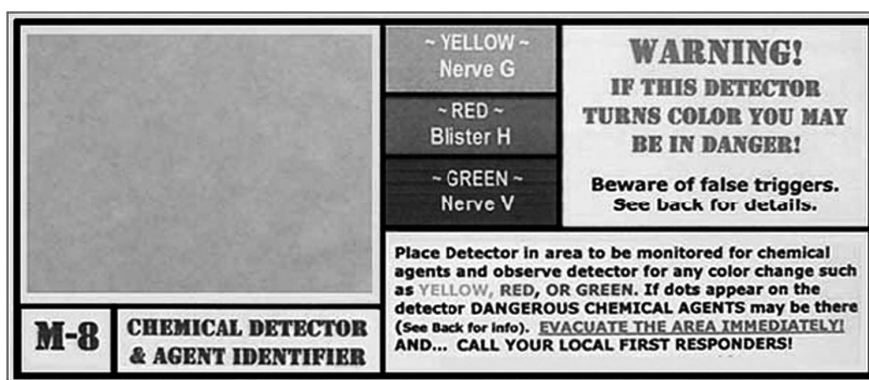


Figure 1. M8 detection paper. (Reproduced by permission of Anachemia Canada Inc. Photo credit: Two Tigers Radiological)

agent. However, 'false positives' can occur if liquid pesticides, antifreeze, or petroleum are present in the tested agent(s). The M9E1 tape comes in an adhesive-backed roll, which can be worn on the uniform, and detects the presence of liquid V- and G-type nerve agents and H- and L-type blister agents. However, detection papers are not as reliable as other means of detection because they depend on liquid agent contacting the surface of the paper; neither type of paper detects traces of chemical agent vapors. Anachemia provides a chemical agent vapor detector product for that purpose. Some solvents and standard decontaminating solutions cause false-positive reactions with the M8 paper. Extremely high temperatures, scuffs, certain types of organic liquids, and decontaminating solution # 2 can cause false-positive reactions with the M9 paper. The M9 paper does not distinguish between the types of agent involved, only that an agent or agents may be present. Similar detector paper booklets, type X-1 and X-3, are in current service with the Chinese armed forces.

2.2 Detection Tubes

In these systems, a small manual air pump draws air through a tube impregnated with an indicator that absorbs agent. The tube may require the addition of a developer for verification. Tubes are provided by several vendors, most prominently Dräger (Figure 2), Gastec, and RAE Systems. The reagents are usually redox active and are class sensitive, not specific to individual agents. Many Dräger tubes indicate easily oxidizable molecules by the reduction of I_2O_5 to I_2 . In another example, the Dräger phosphoric acid ester tube contains the enzyme cholinesterase, so as to imitate nature. But, in this case, inhibition of the cholinesterase results in a color change from yellow to red, as indicated by phenol red. While this tube can reliably detect nerve agents, it will also respond to other cholinesterase inhibitors such as pesticides. In addition to the Dräger tubes, AFC International also makes the chip measurement system (CMS), which utilizes the same color-changing-reaction technology as the tubes, but in a 'chip' format. The system combines the color-changing chip with an optical reader, a mass flow controller, and a pump into a lightweight (0.7 kg), handheld system ($20.3 \times 9.1 \times 4.3$ cm).

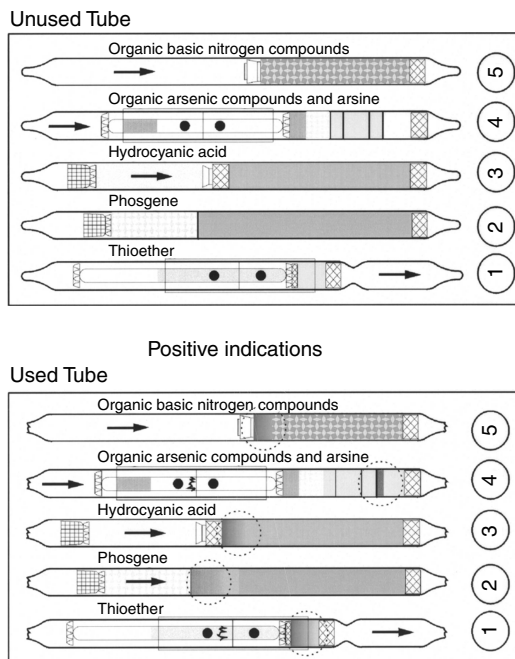


Figure 2. Detection tubes instruction cards (from Draeger Safety)

2.3 Detection Kits

The M256A1 Chemical Agent Detector Kit is a portable, disposable chemical agent detector kit that can detect and identify nerve, blister, or blood agent vapors. It is typically used to determine when it is safe to unmask after a chemical agent attack. Each kit consists of 12 disposable sampler detectors, one booklet of M8 paper, and a set of instruction cards attached by a lanyard to a plastic carrying case. The case is made from molded, high-impact plastic, with a nylon carrying strap and a nylon belt attachment. Each sampler detector contains a square, impregnated spot for blister agents, a circular test spot for blood agents, a star test spot for nerve agents, and a lewisite-detecting tablet and rubbing tab. The test spots are made of standard laboratory filter paper. There are eight glass ampoules, six containing reagents for testing and two in an attached chemical heater. When the ampoules are crushed between the fingers, channels formed in the plastic sheets direct the flow of liquid reagent to wet the test spots. Each test spot or detecting tablet develops a distinctive color that indicates whether

a chemical agent is or is not present in the air. The M18A2 Chemical Agent Detector Kit (currently called ABC-M18A3) is aimed at both the collection and identification of chemical weapons (CW) agents, as well as toxic industrial chemicals (TICs). The kit contains sealable sampling tubes for the safe transport of unidentified, but suspect, samples to approved laboratories. It uses both detector tubes and paper tickets to detect and classify dangerous concentrations of lethal chemical agents in the air, as well as liquid chemical agent contamination on exposed surfaces. Agents detected are as follows: cyanogens chloride (CK), sulfur mustard (HD), nitrogen mustards (HN-1 and HN-3), phosgene oxime (CX), hydrogen cyanide (AC), phosgene (CG), Lewisite (L), ethyl dichloroarsine, methyl dichloroarsine, the G-series nerve agents, and VX (*O*-ethyl-S-(2-isopropylaminoethyl) methylphosphorothiolate). At present, there are no packaged detection kit systems for the arsenical vomiting agents, tear gases, and incapacitating agents. The use of an eel enzyme for the nerve test in place of the horse enzyme provides for an improvement to the M256A1 Kit by detecting lower levels of nerve agent. Any type of mustard is also detectable as long as vapor is present. Both of these kits are available from Anachemia; the former is sold as the Chemical Agent Detector Kit C-2 (Figure 3).

All of these individual detection devices are simple, very sensitive, and thus give a very rapid response. The drawback to the simplicity is that these devices generally do not identify the specific agent that is present, only the class of agent; the

drawback to the sensitivity is that these devices can give false-positive readings. Therefore, separate and orthogonal systems are needed to verify the presence of an agent and to identify the specific agent. Many military organizations rely on manually operated colorimetric tests as a first indicator of the presence of chemical agents. Such tests are impractical for continuous, unattended monitoring.

3 POINT DETECTION INSTRUMENTS

Most of the instruments and devices available for detecting chemical weapons are for point detection. There are three major types of point detection instruments: ion mobility spectrometers (IMS), gas chromatographs (GCs), or surface acoustic wave sensors (SAWS). Normally, these devices are designed to draw in air directly from the atmosphere, but some have the option of using sorbent tubes and other concentrating methods. Major considerations are weight, power requirements, ease of operation, data logging, and false-negative/positive responses.

3.1 Ion Mobility Spectrometry

The most common device used for chemical monitoring by the military is the IMS. The applications of IMS were detailed in a recent review ^(1,2). Essentially, the device functions by measuring the transit time of ions as they pass through a drift region under the influence of an electric field at ambient pressure. This transit time is indicative of a particular substance based on its molecular mass and geometry, with geometry becoming more important as mass increases. The ions are detected electrically. Owing to the collisions with gas molecules in the drift region, the widths of the analyte peaks are large relative to the total analysis time. Because ions are admitted in pulses on the order of microseconds, and repetition rates are less than 30 Hz, it takes only seconds to get enough averages for a spectrum. This results in a reasonable response time but the low duty cycle reduces sensitivity. Although reports of the separation of geometric isomers makes the technique appear to be a high-resolution method, in reality the typical width of the peaks leads to overlaps for similar compounds. To reduce this problem,



Figure 3. Chemical agent detection kit C-2. (Reproduced by permission of Anachemia Canada Inc.)

the selectivity of IMS is typically adjusted by the type of ionization method employed and the use of a particular reagent gas. The proper reagent chemistry can allow more selective detection of a group of compounds. But, the best route to unambiguous identification is the combination of IMS with some form of GC. This increases the analysis time but reduces ambiguity. The current state of the art incorporates high-resolution IMS with GC and a mass spectrometer (GC/HRIMS/MS- as presented by Herbert Hill – Washington State University).

3.1.1 IMS Examples

The GID-3 is a British-made IMS for battlefield detection of the most common chemical agents. The GID-2A is a similar device designed for fixed locations and unattended operation. It is very portable and rugged. In the United States, it is known as the XM22 ACADA (Figure 4). The related chemical agent monitor (CAM) handheld unit operates on



Figure 4. The Automatic chemical agent alarm (ACADA) system, also known as GID-3

similar principles and is mass-produced (>57 000 units). The latest version for the United States is called the improved chemical agent monitor (ICAM) and is marketed by Intellitec (13 000 units). Currently, Smiths Detection manufactures both of the CAM and E-CAM devices, as well as the graseby ionics detector (GID) devices.

3.1.2 GID Specifications

Detection: GID-3 is reported to detect the most common nerve and blister agents and can be programmed to detect other agents such as blood, choking, and chlorine gas. **Dimensions:** 27.30 cm × 17.78 cm × 16.51 cm (detector); 7.6 cm × 17.78 cm × 15.24 cm (battery box and battery); 409 cm × 329 cm × 200 cm (vehicle mount). **Weight:** 4.8 kg (detector); 1.6 kg (box and battery). The operating temperature range of GID-3 is between -30°C and $+50^{\circ}\text{C}$, and storage between -40°C and $+70^{\circ}\text{C}$. The GID-3 has successfully undergone extensive environmental testing against damage due to shock, vibration, electromagnetic pulse (EMP), and electromagnetic compatibility (EMC), both for operating and for storage. The GID-3 is reported to be resistant to false alarms, driving rain, dust, and sand. **Main power supply:** 115–220 VAC, when using the GID-3 power supply; **battery power supply:** 24 V lithium sulfur dioxide battery. Typical battery life is 14 h of continuous usage (at 20°C). A communications port provides data output in a RS-232 format.

Chemical Agent Monitor (CAM). A handheld ICAM (also known as CAM2) is a portable IMS. An Enhanced Chemical Agent Monitor (E-CAM) is also available from Smiths Detection. Air is drawn into the IMS unit and is ionized by a weakly radioactive source. A computer examines the pattern of the time(s) of flight in a sample, determines the level of chemical agent present, and indicates on a display – or by an alarm – the level of hazard. Additional programming can be included to extend the range to cover other agents and certain harmless chemical simulants used as training aids. The levels of detection for the default agents are in Table 1. The unit is powered by a single 6 V battery (sealed Li/SO_2 system), which typically lasts for 14 h with continuous usage (at 20°C). **Temperature Range:** -30°C to $+50^{\circ}\text{C}$ operating (-50°C to

Table 1. Some military chemical weapons detectors.^a Supplemented by manufacturers' specifications

Equipment	Agent	Sensitivity	Time	Cost	Operational or maintenance/limits	Notes
M8 Paper	Nerve-G	100- μ drops	<30 s	\$1 per/book 25 sheets	Disposable/handheld, Dry undamaged paper	Chemical agent detector, potential for false positives
	Nerve-VX	100- μ drops				
	Mustard-H	100- μ drops				
M9 Paper	Nerve-G	100- μ drops	<20 s	\$5 per 10-m roll	Disposable/handheld 3-Year shelf life	Adhesive-backed dispenser roll or books
	Nerve-VX	100- μ drops				
	Mustard-H	100- μ drops			Carcinogen	
M18A2 Detector kit	Liquids only					
	Nerve-GB	0.1 mg m ⁻³	2–3 min	\$360	Disposable tubes handheld	25 Tests per kit; Detector tubes, detector Tickets, and M-8.
	Nerve-VX	0.1 mg m ⁻³				
	Mustard-H, HN, HD, HT	0.5 mg m ⁻³				
	Lewisite-L, ED, MD	10.0 mg m ⁻³				
	Phosgene-CG	12.0 mg m ⁻³				
	Blood-AC	8.0 mg m ⁻³				
	Liquid, vapor, aerosol					
	Nerve-G and VX	0.005 mg m ⁻³	15 min Series is Longer AC- 25 min	\$140	Disposable Handheld 5-year shelf life	Each kit contains 12 Disposable, plastic sampler-detectors and M8 paper.
	Mustard-HD	0.02 mg m ⁻³				
	Lewisite-L	2.0 mg m ⁻³				
	Phosgene oxime-CX	9.0 mg m ⁻³				
M272 water test kit	Blood-AC, CK	3.0 mg m ⁻³	7 min 7 min 7 min 6 min	\$189	Portable/lightweight 5-year shelf life USN; USMC	Used to test raw or treated water; Type I and II detector tubes, eel enzyme detector ticket; Kit conducts 25 tests for each agent
	Vapor or liquid	8.0 mg m ⁻³				
	Nerve-G and VX	0.02 mg m ⁻³				
	Mustard-HD	2.0 mg m ⁻³				
	Lewisite	2.0 mg m ⁻³				
	Hydrogen cyanide	20.0 mg m ⁻³				
CAM Chemical Agent Monitoring	Nerve-GA, GB, VX	0.03 mg m ⁻³	30 s \leq 1 min	\$7500	Handheld/portable battery operated 6–8 h continuous use. Maintenance required.	Radioactive source. False alarms with perfume, exhaust paint, additives to diesel fuel.
	Blister-HD and HN Vapor only	0.1 mg m ⁻³				

ICAM Improved Chemical Agent Monitor	Nerve-G and V Mustard-HD	0.03 mg m ⁻³ 0.1 mg m ⁻³	10 s 10 s	\$7500	4.5 Pounds Minimal training	Alarm only. False positives common. Audible and visual alarm.
ICAM-APD Improved Chemical Agent Detector-Advanced Point Detector	Nerve-G Nerve-V Mustard-H Lewisite-L	0.1 mg m ⁻³ 0.04 mg m ⁻³ 2.0 mg m ⁻³ 2.0 mg m ⁻³	30 s 30 s 10 s 10 s	\$15 000	12 Pounds including batteries Low maintenance Minimal training	Audible and visual alarm.
ICAD Miniature Chemical Agent Detector	Nerve-G Mustard-HD Lewisite-C Cyanide-AC, CK Phosgene-CG	0.2–0.5 mg m ⁻³ 10 mg m ⁻³ 10 mg m ⁻³ 50 mg m ⁻³ 25 mg m ⁻³	2 min (30 s for high levels) 2 min 15 s	\$2800	8 oz Pocket-mounted 4 Month service No maintenance Minimal training.	Audible and visual alarm. Marines; No radioactivity.
M-90 D1C Chemical Agent Detector	Nerve-G, V Mustard Lewisite Blood Vapor only	0.02 mg m ⁻³ 0.2 mg m ⁻³ 0.8 mg m ⁻³	10 s 10 s 80 s	\$16 000	15 lb. with battery Radioactive source exempt from licensing. Minimal training	Ion mobility spectroscopy and metal conductivity technology can monitor up to 30 chemicals in parallel. Alarm only.
M8A1 Alarm Automatic Chemical Agent Alarm	Nerve-GA, GB, GD Nerve-VX Mustard-HD Vapor only	0.2 mg m ⁻³ 0.4 mg m ⁻³ 10 mg m ⁻³	≤ 2 min ≤ 2 min ≤ 2 min	\$2555	Vehicle battery operated Maintenance required	Radioactive source (licensing required); Automatic unattended operation; Remote placement
MM-1 Mobile Mass Spectrometry Gas Chromatograph RSCAAL M-21 (a/k/a JSLSCAD)	20–30 CWA Vapor Nerve-G Mustard-H Lewisite-L Vapor	< 10 mg m ⁻² of surface area 90 mg m ⁻³ 2300 mg m ⁻³ 500 mg m ⁻³	≤ 45 s	\$300 000 military \$100 000 civilian \$110 000	Heater volatilizes surface contaminants. Line-of-sight dependent 10 year shelf life 2-person portable tripod	German 'Fuchs' (FOX Recon System/Vehicle) Passive infrared energy detector 3 miles; Visual/audible warning from 400 meters

(continued overleaf)

Table 1. (continued)

Equipment	Agent	Sensitivity	Time	Cost	Operational or maintenance/limits	Notes
SAW MiniCAD	Nerve-GB	1.0 mg m ⁻³	1 min	\$5500	Minimal training	Alarm only; False alarm from gasoline vapor, glass cleaner.
	Nerve-GD	0.12 mg m ⁻³	1 min		Field use	
	Mustard-HD Vapor	0.6 mg m ⁻³	1 min		1 pound No calibration	
ACADA (XM22, GID-3)	Nerve-G	0.1 mg m ⁻³	30 s	\$8000	Vehicle mounted, battery powered.	Radioactive source (license required) Minimal training
	Mustard-HD Lewisite Vapor	2 mg m ⁻³ —	30 s —			
Field MiniCAMS™	Nerve-G, V	<0.0001 mg m ⁻³	<5 min	\$34 000	Designed for field industry monitoring (10 lb.)	8 h training 24 h/7 day
	Mustard-HD	<0.003 mg m ⁻³	<5 min			
	Many others	<0.003 mg m ⁻³	<5 min			
Viking 573 GC/MS (Bruker Daltonics)	Nerve-G, V	<0.0001 mg m ⁻³	<10 min	\$100 000	Field use, but 85 pounds	Needs 120 VAC, 40 h training
	Mustard-HD Many others	<0.003 mg m ⁻³	<10 min			
Agilent 6890 GC with flame photometric detector (FPD)	Nerve-G, V	<0.0001 mg m ⁻³	<10 min	\$50 000	Designed for lab use	Gas, air, 120 VAC 40 h training
	Mustard-HD Many others	<0.0006 mg m ⁻³	<10 min			

^a Adapted from reference 1.

+70 °C storage). Dimensions: 38 cm longest dimension. Weight: 1.9 kg, hand unit with battery.

ACADA replaces the M8A1 Automatic Chemical Agent Alarm System, which was used during the 1991 Gulf War. The M8A1 is a portable chemical agent alarm, also based on IMS. Description: The ACADA is a higher-resolution IMS. The unit eliminates false positives through the use of dual 4-inch long analysis cells, somewhat longer than the competing Graseby technology, which uses 2.1-inch long cells. The dual cells are used to examine positive and negative ions simultaneously. The unit can make some detections within a few seconds of exposure and can clean itself out for a fresh detection within 60 s. Its sensitivity to nerve agents is 0.1 mg/m³ and to blister agents is 2.0 mg/m³ (roughly corresponding to 0.1 ppb and 2.0 ppb). At these levels, the unit is designed to register 'no false positives' to shipboard contaminants, such as paints, oils, and flame retardants. It samples 4 L of air per minute without a preconcentrator. The unit is roughly 45.7 × 15.2 × 15.2 cm, contains an RS-422 serial port connection, and runs on 12 V DC or 110 V AC power. It is an upgrade to the Improved Chemical Agent Point Detection System (IPDS) currently in use by the US Navy.

3.2 Gas Chromatographs

The military primarily uses GC to monitor storage sites. GC, when combined with mass spectrometry (GC/MS), is specified by the Organization for the Prohibition of Chemical Weapons (OPCW) as the analytical method of choice when testing samples at suspected sites of chemical agent contamination. The principles of GC and the specific detectors employed will be discussed more thoroughly in the section on commercial systems. Two particular detection technologies are used at chemical warfare agent depots and demilitarization plants. The first of these systems is similar to the point detection systems, in that it is automated, rapid, and sensitive. The second system is used to verify the results obtained with the first; it requires sampling and subsequent analysis at an on-site laboratory.

The Miniature automatic Continuous Agent Monitoring System (MiniCAMS™) utilizes an automated near-real-time gas chromatograph. An air sample is drawn through a preconcentrator loop filled with an

adsorbent. Periodically, the system is switched so that a carrier gas stream flows through the preconcentrator loop as it is heated, carrying the adsorbed sample into the GC. The GC separates the chemical compounds in the sample based on differential partitioning between the carrier gas (usually nitrogen, helium, or hydrogen) and the stationary phase in the GC column. While the sample components are being separated, the preconcentrator loop begins to collect the next sample. MiniCAMS™ detects agents with a Flame Photometric Detector (FPD), which specifically detects the chemiluminescent reactions of compounds containing sulfur and phosphorus in a hydrogen/air flame. The entire cycle from sample collection to detection typically requires 3–5 min. MiniCAMS™ is a refinement of the larger Automatic Continuous Agent Monitoring System (ACAMS) detector; ACAMS is still in use in some locations. The commercial form of this instrument is available from O.I. Analytical and the latest version is called the FM-3000 (Figure 5).

The Depot Area Air Monitoring System (DAAMS) is a system used to confirm the detection of an agent by the MiniCAMS™. Larger air samples are drawn continuously through the DAAMS preconcentrator tube, which contains a correspondingly larger quantity of adsorbent. At either a predetermined time, or when a confirmation of a MiniCAMS™ result is required, the DAAMS tube is physically transported to the laboratory, where the sample is desorbed into a laboratory gas chromatograph.

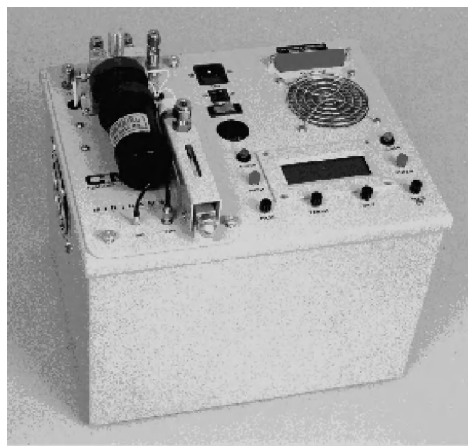


Figure 5. FM-3000 (from O.I. Analytical)

3.3 Surface Acoustic Wave Sensors

SAWS devices work in the same manner as do Quartz Crystal Microbalances (QCMs), but at higher frequencies and with greater sensitivities. One example is the SAWRHINO. This is a SAWS-based detector that incorporates a separation step by first collecting vapors through condensation and then desorbing the vapors over a 3-SAW detector to give a time versus temperature trace. In this way, the detector combines the separation aspects of GC with the detection and identification capabilities of a multi-SAWS detector. As with most SAWS devices, the detection of chemical agents relies on the application of pattern recognition algorithms. The SAWSRHINO system uses the responses generated from the three variously coated SAWS devices as the input data for a neural network algorithm to determine a recognition pattern. The military SAWS devices include the SAWCAD, MiniCAD and, as will be discussed later, the JCAD, which combines the SAWS detector with an electrochemical cell.

3.4 Commercial Devices

As stated above, most of the military devices are now available in a commercial version. This section will primarily discuss existing commercially available devices that could be applied to chemical agent detection but are not necessarily marketed for that application.

3.4.1 Gas Chromatography

All of the detection methods below benefit greatly from a separation of components prior to detection. GC is a very efficient separation technique that generally allows separation of analytes from background species. The detection methods outlined above can be applied to not only detect species but also to some degree discriminate against types of compounds that might normally interfere. The problems associated with current gas chromatographs, with respect to continuous monitoring, are size and speed. These problems could be addressed by appropriate redesign and miniaturization. Gas chromatography will mate with the following detectors.

3.4.2 Thermal Conductivity Detector (TCD)

Traditionally, the TCD has been recognized as a universal detector, but one of limited sensitivity. Several manufacturers have invested in miniaturization of the TCD, with improvements in sensitivity, resulting in lower detection limits. For example, Varian sells a multichannel GC (CP-4900 Micro GC) incorporating a MEMS-based TCD with the unrivaled sensitivity of about one ppm.

3.4.3 Electron Capture (EC)

The EC detector is designed to detect molecules with highly electronegative elements such as halogens. The electrons are typically provided by a radioisotope like ^{63}Ni or tritium (^3H) absorbed into a palladium host. The presence of compounds with highly electronegative elements – such as G and V agents – can be detected at extremely low levels. Again, high selectivity would be dependent on a prior separation.

3.4.4 Thermionic Detector

The thermionic or alkali flame ionization detector (FID) is a variant of the FID that has enhanced sensitivity to nitrogen (N) and phosphorous (P) containing compounds. It is essentially a hydrogen flame around a pellet of an alkali metal salt with an electric potential across the flame. Organic compounds in the air are drawn into the flame and ionized. The alkali metal enhances the response to N and P-containing compounds and, since many chemical agents contain N and P, it is selective for their detection. This detector is rarely used without prior separation by GC.

3.4.5 Flame or Low Energy Plasma Photometry

Most chemical agents contain specific elements in common. G and V agents all contain phosphorus and blister agents, like the mustards, contain sulfur or nitrogen. The combustion of these materials yields excited atoms that emit light characteristic of these elements. The emissions are viewed through an interference filter by a photodetector. As an alternative to a flame, low-powered, inductively coupled (or microwave) plasmas have been used as emission sources. These sources have been combined with

photodiode array detectors and are called atomic emission detectors (AED). This allows the detector to acquire a full spectrum, which gives information on most of the elements in a compound. The combination of GC and an AED can often provide an approximate empirical formula. This type of detector was previously marketed by Hewlett Packard but is now available from Joint Analytical Systems.

3.4.6 Photoionization

The presence of organic compounds in air can be detected by using a light source with sufficient energy to ionize the organic compounds, resulting in gas electrical conductivity. Selectivity of detection is obtained by tuning the light source to the right energy. Unfortunately, all compounds with ionization energies lower than that supplied by the lamp will be detected. This method then becomes reliant on a separation step (i.e. GC) to give good identifications (see Table 2).

3.4.7 Mass Spectrometry (MS)

MS has long been used for the detection of low molecular weight gases. Hence, the devices are sometimes called residual gas analyzers and are typically magnetic sector or quadrupole instruments. The mass spectrometer is often considered to be the best GC detector since it can usually provide unambiguous identification of eluted components. A variety of mass spectrometers have been used as

GC detectors, with the quadrupole mass selective detector (MSD) the most popular. Quadrupole mass spectrometers are selective ion filters that can scan a suitable range of mass/charge (m/z) ratios in milliseconds. The popularity of the quadrupole is due to its economy, speed, and ability to tolerate higher pressures than other mass spectrometers, making it especially suitable as a detector for GC. Quadrupole mass spectrometers are tuned so that the spectra produced are similar to the spectra obtained from a magnetic sector mass spectrometer, allowing both types to use the same spectral libraries for compound identification. The quadrupole mass spectrometer is usually the analyzer of choice for gas analysis since it can tolerate higher pressures than other analyzers. The primary use for these devices is to detect (organic) molecules that in application of electron impact (EI) ionization produce fragmentation of the molecule. This approach can be applied to chemical agent detection with the addition of detection algorithms designed to recognize the fragmentation pattern of the agents. Unlike FT/IR, mass spectrometers can scan rapidly; so, fast GC methods can be used with these detectors. Tandem MS is an approach that uses one mass spectrometer to select a specific ion from a sample to be further fragmented and mass analyzed by a second mass spectrometer. Since more qualitative data is produced, this method may be used in the future as algorithm developments progress.

Mass spectral interferences are a function of the method of ionization. The standard ionization method is EI ionization, or simply electron

Table 2. Properties of common GC detectors

Detector	Principle of operation	Selectivity	Sensitivity (gm/sec)
Flame ionization	H ₂ -O ₂ Flame 2000 °C Plasma	Responds to organic compounds not to fixed gases or water	9×10^{-16}
Electron capture ³ H ⁶³ Ni	N ₂ + $\beta^- \rightarrow e^- e^- +$ sample \rightarrow loss of ion	Response to electron adsorbing compounds (especially halogens, nitrates and conjugated carbonyls)	2×10^{-14} for CCl ₄ 5×10^{-14} for CCl ₄
Thermionic P-containing compounds N-containing compounds	Alkali modified H ₂ -O ₂ Flame 1600 °C Plasma	Enhanced response to phosphorus compounds and nitrogen compounds	4×10^{-14} 7×10^{-12}
Photoionization	He + $\beta^- \rightarrow$ He* sample He* > 10	Universal – responds to all compounds	2×10^{-14} for methane
AED	Flame or plasma	Universal – responds to all compounds	1×10^{-9}

ionization. This method results in extensive fragmentation of the sample molecules and may eliminate the parent ion completely. While the fragments can be used as a fingerprint to help identify a compound, this requires an extensive database and computer support. Such systems are currently beyond the scope of commercially available on-line chemical sensors. One alternative to EI ionization is the use of chemical ionization (CI). This reduces fragmentation and increases sensitivity by preserving the molecular ion. Chemical ionization simplifies identification but complicates the hardware system, since a cylinder of reagent gas is required (e.g. methane, isobutane, ammonia). The combination of GC and MS can usually provide the selectivity needed for unambiguous identification.

At least two companies now have 'portable' GC/MS systems that are commercially available and intended for hazardous area monitoring – Bruker Daltonics (Viking 573) and Constellation Technology (CT-1128, Figure 6). Both systems have essentially miniaturized the lab-based systems and made them more robust for transport and field use, although still requiring ~120 VAC power. Both systems make use of Agilent's 5973 MSD and utilize Agilent's ChemStation software to leverage the knowledge and experience of proven detection and software systems. The CT-1128 comes in a transportation box with dimensions of 53 cm L × 35.6 cm H × 38 cm W and a weight of 165 kg. Two other MS detectors used for GC are the quadrupole

ion trap and the time-of-flight mass (TOF) spectrometer. These systems are available for laboratory use but no portable instruments are yet available. A fast TOF-based GC/MS is available from Thermo Finnegan called the TEMPUS.

3.4.8 Surface Acoustic Wave Sensors (SAWS)

The SAW MiniCAD is a commercially available (Microsensor Systems, Bowling Green, KY), pocket-sized instrument that can automatically monitor for trace levels of toxic vapors of both sulfur mustard and the G-type nerve agents with a high degree of specificity. The instrument is equipped with a vapor-sampling pump and a thermal concentrator to provide enriched vapor sample concentration to a pair of high-sensitivity-coated SAW microsensors. All subsystems are designed to consume minimal amounts of power from onboard batteries. Optimal use of the SAW MiniCAD requires that a suitable compromise be made among the conflicting demands of response time, sensitivity, and power consumption. Maximum protection requires high sensitivity and a rapid response. The SAW MiniCAD is able to achieve a high sensitivity with an increased vapor-sampling time. However, a faster response can be achieved at a lower sensitivity setting. Testing of the SAW MiniCAD has been performed with chemical warfare agents such as soman, tabun, and sulfur mustard gas. These tests were performed at a variety of concentrations and humidity levels. There were no significant effects noted because of the changes in the humidity levels for any of the chemical agents tested.

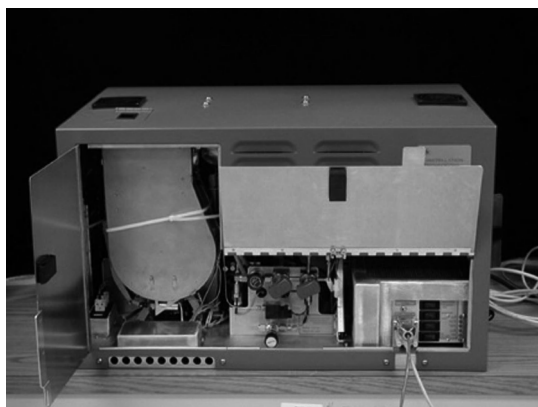


Figure 6. Constellation technology (CT-1128)

4 STANDOFF DETECTION INSTRUMENTS

The M21 remote sensing chemical agent alarm (RSCAAL) is based on a passive infrared (IR) detector. The incoming IR signal is compared against known agent spectra; when a match is detected, a display lights and an alarm sounds. The display also indicates in which of the seven fields of view (spread over a 60 degree arc) the agent was detected. This allows the operator to actually track a moving agent cloud. The M21 is capable of

detecting nerve and blister agents in the vapor phase from a distance of up to 5000 meters; however, it must have a direct line of sight to the agent cloud in order to function. It is being replaced by a newer version, the joint service lightweight standoff chemical agent detector (JSLSCAD, available from General Dynamics Armament and Technical Products division). The unit is a passive FTIR (Fourier-transform infrared) spectrometer with 360° coverage in a 60° cone angle and a 5 km range, less in humid or obscuring conditions. The unit utilizes the highly sensitive HgCdTe detector, which has had some difficulties due to the need to cool the spectrometer. It detects nerve, blister, and blood agent vapor clouds in unattended operation, using automatic warning and reporting through the joint warning and reporting network (JWARN) system. A commercial, passive-IR detector called remote air pollution infrared detector (RAPID) is available from Bruker Daltonics Inc. (Billerica, MA, USA). The RAPID was designed for mounting on a vehicle. Its size is relatively small, (50 × 33 × 39 cm), relatively light (28.7 kg) and touts an easy-to-use, Microsoft® Windows-based user interface. The system runs on 18–36VDC power and scans a complete 360° rotation in 3 sec. Light detection and ranging (LIDAR) and differential absorption LIDAR (DIAL) systems are still under development.

5 RESEARCH AND DEVELOPMENT

5.1 Military Research and Development

The critical need for improved hazardous chemical sensors has spurred a large investment in research and development by many nations and supranational groups such as North Atlantic Treaty Organization (NATO). This investment is apparent by the large number of different devices currently under development. While the military is either performing or sponsoring many developments in chemical agent detection, their investment has focused more on engineering and less on innovation. The common theme is a 'joint effort,' aimed at consolidation of technologies from all branches of the US military. However, many of these projects are undeveloped or based on incremental increases in technology and

cannot be expected to yield much improvement in the near term; civilian-sponsored investigations are far more likely to make major breakthroughs in technology in the near term.

Automatic Chemical Agent Detection Alarm (ACADA-M22). The M22 is an advanced, point-sampling, chemical agent alarm system employing ion mobility spectrometry. It is designed to detect standard nerve and vesicant agents.

Improved chemical agent point detection system (IPDS). The IPDS also employs ion mobility spectrometry and is an improved version of a point detection system. In addition to G and VX nerve agents, the IPDS is designed to detect vesicant agent vapors. Because it is a shipboard instrument, it will be much larger and will need more power than portable IMS devices.

Joint chemical agent detector (JCAD). The JCAD will employ surface acoustic wave (SAW) technology to detect nerve and blister agents. It is designed to be lightweight and portable and will reduce false alarms. The JCAD will also allow detection of new forms of nerve agents.

Joint CB Agent Water Monitor (JCBAWM). The JCBAWM will be a portable device to detect, identify, and quantify both chemical and biological agents in water. It will allow the user to sample water and receive a digital readout of the contents. The technology to be employed in this monitor is still under review.

Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD). The JSLSCAD is a passive, IR detection unit employing FTIR Spectrometry. The device is designed to detect nerve and blister vapor clouds at a distance of up to 5 km.

Shipboard automatic liquid agent detector (SALAD). The technologies to be used in the SALAD have recently been reviewed, but no decision has been made on the final selection. The instrument is designed to be an automated, externally mounted liquid agent detector capable of detecting G-type and VX nerve agents as well as vesicant chemical agents.

The Special operations forces (SOF) Non-intrusive Detector and the Swept Frequency Acoustic Interferometry (SFAI) detector are portable, hand-held acoustic instruments developed specifically to enable rapid detection and identification of CW agents within munitions, railcars, ton containers, and so on.

5.2 Civilian Research and Development

A wide variety of approaches to hazardous chemical detection are being investigated with a potential for hazardous environment monitoring. A sample, of some of the more promising approaches, is outlined below. While many of the methods being investigated are only suitable for use in the laboratory at the present time, they might be rapidly converted to field monitors through miniaturization.

5.2.1 Capillary Electrophoresis

Capillary electrophoresis (CE) has been used by several laboratories to analyze mixtures of chemical agents. CE is an attractive approach due to its high separation efficiency and fast analysis time. The difficulty in applying CE to chemical weapons detection is twofold. First, there is a lack of selective detectors for use with CE. Most CE detection is done by UV absorbance, either directly or indirectly, by including a UV absorbing additive to the running buffer. Direct UV detection at 200nm was used by Cheicante *et al.* to determine sulfur containing CW agents using a variant of CE called micellar electrokinetic capillary chromatography (MECC) ⁽³⁾. Mercier *et al.* ⁽⁴⁾ used indirect photometric detection to analyze nerve agent decomposition using indirect UV detection, while Nassar *et al.* ^(5,6) used a similar approach for aqueous solutions and soil extracts with analysis times as short as 3 min. The problem with nonselective detection will likely be addressed by the application of selective GC detectors such as the pulsed FPD. The second issue is a lack of automated CE systems. This is an area of very active research. Groups at Sandia and Oak Ridge National Laboratories are laboring to make miniature CE devices in the 'lab on a chip' format. Again, the incorporation of a selective detector will be needed, but the CE on a chip can easily be made in an array with multiple detectors for unambiguous identification.

5.2.2 He or Ar Afterglow Detection

Inductively coupled plasma emission spectrometry is a standard method for trace elemental analysis. While sensitive and selective, these instruments are large and require considerable support solutions

and gases. Fortunately, there exists a lower power version of this method that is available in the form of a GC detector called the atomic emission detector. In this instrument, a low power plasma is either inductively coupled or microwave induced in an Ar or He gas stream. Compounds are decomposed into their atomic constituents and the emission of the atoms is measured by a spectrometer equipped with an array detector. The commercial version of this detector was developed by Hewlett Packard and is called the atomic emission detector (AED). It is currently being marketed by Joint Analytical Systems. This detector is useful for reducing ambiguities in the Gas Chromatographic (GC) analysis of chemical weapons because the multielement capability gives a rough empirical formula for each peak detected. Creasy *et al.* used Gas Chromatographic-atomic emission detector to analyze environmental samples for CW related compounds ⁽⁷⁾.

5.2.3 Ion Chromatography

Ion chromatography can be used to measure ionic concentrations in solution. It requires a fairly large amount of solvent and usually a suppressor column to neutralize the acidity prior to detection by conductivity. For analytical purposes, most analyses previously performed by ion chromatography are now performed using capillary electrophoresis. However, Katagi *et al.* used ion chromatography with indirect photometric detection to analyze for nerve agents and hydrolysis products in human serum ⁽⁸⁾.

5.2.4 Molecularly Imprinted Polymer Sensors

The process of molecular imprinting creates a selective binding site for a specific molecule ⁽⁹⁾. The site is created by using the molecule of interest as a template in a copolymerization process. The removal of the template leaves a cavity that is selective for rebinding the template molecule. The process of molecular imprinting can be used to improve the selectivity of many existing sensors that rely on selective coatings such as QCM and SAWs. However, the inclusion of a chromophore in the imprinted site can lead directly to an optical sensor for the molecule of interest. If the optical sensor is based on a form of luminescence, highly selective and sensitive sensors can be produced.

Jenkins *et al.* produced a fiber optic based luminescence sensor designed to measure a hydrolysis product of the nerve agent soman (GD) in water. The sensor exhibited high selectivity, no interference from organophosphorous (OP) herbicides or pesticides, and high sensitivity, with a limit of detection of 600 fg/mL in water ⁽¹⁰⁾.

5.2.5 Cavity Ring Down Spectroscopy

This is a method of measuring small amounts of optical absorbency that can be performed with a pulsed light source and that has a significantly higher sensitivity than obtainable in 'conventional' absorption spectroscopy. The cavity ring down (CRD) technique is based upon the measurement of the rate of absorption rather than the magnitude of absorption of a light pulse confined in a closed optical cavity with a high Q-factor. The advantage over normal absorption spectroscopy results from: (1) the intrinsic insensitivity of the CRD technique to light source intensity fluctuations and (2) the extremely long effective path lengths (many kilometers) that can be realized in stable optical cavities. Recently, the technique has been applied to solid optical resonators with selective coatings ⁽¹¹⁾.

5.2.6 Surface Plasmon Resonance (SPR)

This detector is based on the collective oscillations of the free electron plasma at a metal surface. Typically a prism is coated with a metal film and the film coated with a chemically selective layer. The surface is illuminated by a laser and the amount of material adsorbed by the coating affects the angle of the deflected beam. This platform is theoretically similar in sensitivity to a quartz crystal microbalance. This is another platform whose selectivity is based on the coating. The typical coating is using bound antibodies; thus, this device becomes a platform for immuno-sensors ⁽¹²⁾.

5.2.7 Porous Silicon Interferometer

A device based on a porous silica substrate used as a Fabry-Perot interferometer has been reported as a sensor for organophosphorus nerve agents. The porous silica is coated with a surfactant and a copper hydrolysis catalyst. The mode of operation

is through the loss of finesse of the etalon due to the production of HF by the catalytic decomposition of the agent and subsequent reaction of HF with the silicon surface producing roughening. The device, though novel, only applies to fluorine containing compounds ⁽¹³⁾.

5.2.8 Solid Phase Micro-Extraction (SPME)

Recently, the use of fibers coated with a variety of chromatographic stationary phases has become popular as a means of sample acquisition and preconcentration. These fibers can be used for gaseous headspace sampling or as aqueous solution extractants. They are most commonly applied as sampling devices for GC but are also used as samplers for high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC). The advantages of sampling with SPME for GC are preconcentration and solvent peak elimination. Stuff, *et al.* used SPME air sampling for GC analysis of blister agents ⁽¹⁴⁾.

5.2.9 Biosensors

In general, the term biosensor has come to mean any sensor that uses biomolecules, such as antibodies, for chemical recognition. This form of recognition has been long applied as immunoassays for a variety of compounds. Biosensors can be made using a variety of the measurement platforms described previously. There are limitations when applying biosensors to the detection of chemical warfare agent and toxins due to the inability of the organism used to make the antibodies to survive exposure to the toxic chemical. Additionally, the binding event is often irreversible, making biomolecule recognition difficult to apply to real-time monitoring. A review by Paddle in 1996 lists available biosensors and describes their modes of operation ^(15,16).

5.2.10 Conductive Polymer Sensors

Conductive polymers such as polypyrrole or polyaniline have been applied to sensing vapor and gases. These sensors change their conductivity when exposed to organic vapors because of the effects the vapors have on the availability of charge carriers. One way to obtain selectivity for these materials is

to specifically engineer or dope the polymers with recognition sites. The other approach to selectivity is to make an array of different polymers and measure their relative responses to a variety of compounds. The later approach is one of the methods for making an 'electronic nose.' While the electronic support for this approach to multianalyte sensing is less complex, it is unlikely to be as sensitive as the QCM, SAWS, or SPR devices. One way to enhance the sensitivity of these materials is to use a large surface area. This idea is addressed in the work of Collins and Buckley by using conductive polymer coating for fabrics ⁽¹⁷⁾.

5.2.11 Micro-electromechanical Systems (MEMS)

Under G. E. Spangler, Technispan has used micro-electromechanical systems fabrication techniques to develop miniaturized gas chromatography systems for integration with IMS systems (such as the CAM or ACADA). DARPA has provided funding, although no complete system has been produced. Many classical laboratory techniques are being pursued in the MEMS arena, but so far only pieces of devices have been produced.

5.2.12 Surface Acoustic Wave Sensors

Recently, surface acoustic wave sensor (SAWS) technology have been applied to develop gas sensors for a wide variety of analytes ⁽¹⁸⁾. The key to these sensors' specificity is the production of coatings that selectively bind analyte species ⁽¹⁹⁾. Unfortunately, an immuno-protein, the ultimate in chemical selectivity, is not completely specific to the nerve agent and typically binds it irreversibly. Thus, some additional selectivity factor is needed to make a sensor that is truly specific. One method uses a lanthanide chelate coating to give a thermodynamic affinity for organophosphorus compound coordination ⁽²⁰⁾. A variety of lanthanide chelates have been used in the tests with variable but consistent affinities and sensitivities. The most distinct characteristic shared by all the devices based exclusively on thermodynamic affinities is a clear lack of specificity. While the sensors will detect nerve agents in a sensitive and quantitative manner, any other moiety with a similar chemical functionality will give a similar

or, in the case of many of the current sensors, an overwhelmingly greater response. This is unfortunate since organophosphorus pesticides, such as dichlorvos the active ingredient in many flea collars and fly (No Pest[®]) strips and malathion used in fly sprays and in agriculture, are highly prevalent compounds. Thus, the high probability of a false-positive test renders the current line of sensors less attractive. An emerging technology that could greatly improve the selectivity of polymer coatings for SAWS devices is molecular imprinting ⁽⁹⁾.

6 PRINCIPLE CHEMICAL SOURCES OF SENSOR FALSE ALARMS

The most difficult problems in chemical analysis are those that involve 'real samples.' Real samples are by their nature heterogeneous and complex. The atmosphere is an especially difficult matrix due to the plethora of organic compounds that exist as trace constituents. When this atmosphere is brought inside, the number of trace chemicals is increased. Even the most sophisticated analyst, using state-of-the-art chemical instrumentation, will encounter difficulties in identifying and quantitating a trace chemical. Therefore, building an automated chemical sensor system to perform all of these functions is a daunting task and no perfect solutions are yet available. Obviously, the key to unambiguous trace detection is chemical selectivity.

Chemical selectivity can be obtained in a variety of ways. When the analyte of interest is a trace constituent, laboratory analysis methods inevitably begin with a separation process. The modern chemical separation technique for atmospheric samples is gas chromatography. Gas chromatography is an efficient separations process, but high efficiency requires time. More rapid types of gas chromatography sacrifice efficiency for speed and try to compensate the loss in selectivity by using a selective detector. Consequently, continuous monitoring is impractical and, at best, a periodic sampling is all that can be obtained. Therefore, period must be kept short, if a human response is required. As seen above, a monitor consisting of a gas chromatograph (GC) using a FPD (a detector selective for sulfur and phosphorous) is useful for nerve agents. Still, such a system could be fooled by chemically related

pesticides of similar mass and polarity. However, if the system is used in a facility for storage or destruction of chemical weapons, the high sensitivity of the system will be a useful guard against small leaks or short term events. Thus, each detection system is a compromise determined by the application. Additionally, the combination of GC and FPD is much better than either of the devices used alone. Ideally, these 'criteria of selectivity' should be compounded to the point that no false-positive detection can occur. Multiple criteria of selectivity may be obtained by a single method that has many orthogonal components or by combining two techniques that have a different physical basis for detection. For example, if a selective absorbent is used to collect the sample for the gas chromatography/flame photometric detector (GC/FPD) system the chance of false positives drops dramatically.

The reliable detection of chemical agents requires a high degree of selectivity. Owing to the extreme toxicity of most of these agents, this must be coupled with an ability to detect very small quantities. In most chemical instrumentation, unambiguous identification and the detection of small quantities must meet a compromise. It is for this reason that so many chemical agent detection schemes rely on a combination of techniques or 'hyphenated methods,' such as GC/MS. Unfortunately, many of the hyphenated techniques can be large, cumbersome, and require a skilled operator. Therefore, the 'state-of-the-art' in chemical agent detection typically follows one of two paths: (1) miniaturization and automation of a classical hyphenated technique or (2) the development of new technologies that have an intrinsic selectivity and sensitivity link.

6.1 Methods

Chemical interferences are dependent upon the detection method. This results from the fact that many of the detection methods are not molecule-specific. For example, many of the detection papers change color based on a physical property that many similar chemicals share. Thus, the topic of interferences will be discussed in a method-specific manner. This discussion has been limited to the sensor systems that are candidates for use as continuous real-time monitors.

6.1.1 Surface Acoustic Wave Sensors (SAWS)

As described above, SAWS detectors measure the mass of materials that stick to the surface of the device. These devices are typically used in pairs, with one of the surfaces kept clean to act as a reference. The selectivity of a SAWS device is entirely due to the selectivity of any surface coating. In the case of CW agent detection, the coatings are typically organic polymers. These polymers are designed to have favorable interactions with certain chemical functional groups associated with the chemical agent being sought. The interactions range from weak Van der Waals and dispersion forces to stronger hydrogen bonding. Unfortunately, many chemically similar compounds will also bind to the SAWS. For example, nerve agent sensor SAWS are coated with acidic groups to interact with the basicity of the phosphonate esters. The problem is that not all phosphonate esters are CW agents. This functional group is the basis of most pesticides and many herbicide formulations. Other problems arise from humidity. Since the polymer can hydrogen bond with water, humidity must be measured and its effects subtracted. In the SAWS community, these problems are being addressed in the classical manner of using the chemical separation techniques as described above, or by using an array of SAWS devices with multiple coatings and applying pattern recognition algorithms to help selectivity. The arrays are still subject to interferences from similar compounds and will give false positives. The most recent recommendations are to use SAWS in conjunction with a verification sensor that works by another physical principle, such as IMS (an 'orthogonal technique'). This would greatly reduce the false-positive rate but comes with a much higher expense. SAWS sensors generally suffer from interferences by chemically similar compounds, hydrocarbons, and moisture.

6.1.2 Ion Mobility Spectrometry (IMS)

The selectivity of IMS is dependent on several key steps. The first step in the process is ionization. The process of ionization can be made selective for a class of compounds by either physical or chemical processes. Photoionization is an example of a selective physical means of ionization. Using lamps with differing energies can select against compounds

with lower ionization thresholds. However for IMS, ionization is normally achieved by using a ^{63}Ni radioactive source. The beta radiation electrons from the ^{63}Ni impact the nitrogen in air and create ions. In air, these ions react by a complicated mechanism to eventually produce protonated water clusters from atmospheric moisture. The water clusters react with other gas phase molecules to produce positive ions. A reagent gas (pure chemical) is usually added to the ion source to affect selectivity. For example, nerve agents are basic and accept protons easily. Acetone is added to the ion source to eliminate ionization of compounds that are weaker proton acceptors than acetone. The result is that fewer species are ionized and potential interferences are eliminated.

The next step in the IMS process is the migration of the ions in the electric field. This process is designed to separate the ions based on their ionic mobility. Atmospheric pressure electromigration results in broad peaks due to collisions with the gas molecules in the drift region. Thus, the IMS spectra (plasma chromatograms) may be poorly resolved. This problem is exacerbated by the clustering phenomena noted in the ionization process. Additionally, impurities in the gas (air) that occupies the drift region can do chemistry and cause irreproducibility. This can be eliminated if purified air is used in the drift region, but is impractical for field-deployed devices. Separation occurs in the drift tube based on an ionic mobility that has components of mass to charge ratio and geometry. The geometry component comes from the collisions that occur between sample molecules and the air, thus compounds with the same mass may have significantly different migration times. As a result of these processes, the peak widths seen for laboratory IMS instruments are much better than the field units. Thus, IMS detectors designed for field use are prone to interferences from a variety of sources and exhibit high rates of false-positive and false-negative reports.

IMS can be combined with gas chromatography to give much more reliable detection. Since the separation of chemicals takes time when traditional chromatography is applied, response time becomes a problem. Fast GC, as described above, may be a means to get around this problem. However, IMS does not appear to be a useful detector for fast GC because it is limited to a simple Faraday cup detector by the presence of gas at atmospheric pressure. This

detector is much less sensitive than the detectors employed by traditional gas chromatographs such as mass spectrometers. Probably the best way to employ IMS for the detection of chemical agents would be to use the IMS as a plasma chromatograph coupled to a small quadrupole mass spectrometer equipped with a sensitive electron multiplier or a multichannel plate detector. This should give a rapid response instrument with much greater reliability. To date the only application for IMS-MS has been fundamental studies of atmospheric pressure chemical ionization (APCI).

6.1.3 Gas Chromatography (GC)

Monitors that employ gas chromatography will have a sampling interface that will trap gases and preconcentrate the sample prior to injection. This process is allowed by the periodic nature of the analysis and is matched to the required time for the chromatograph to perform the chemical separations process. A balance must be made between the sample separation processes and the required speed of analysis. The selectivity of the gas chromatograph is tuned by the choice of the stationary phase used in the capillary column. The materials used to coat the capillary are similar to the polymer coatings described for SAWS and other sensors. Temperature programming of the column is not employed in a continuous monitor owing to the time required to cool the column before the next injection.

The ultimate selectivity of gas chromatography is determined by the detector. The most selective detectors are spectroscopic, such as Fourier-Transform Infrared or Mass Spectrometer. Automated systems can employ chemometric algorithms to discriminate unresolved chromatographic peaks. These combinations are expensive and require significant computer support. As such, they are more likely to be used in a laboratory for confirmation. Efforts to convert this approach to field units are still under development. The MiniCAMS[™] described above, based on a FPD is a reliable monitor but requires 3–5 min to make a determination. Gas chromatographs also require a source of purified gas for operation and the flame detector requires additional hydrogen and air for operation. This device will have the fewest false positives and the most

sensitive detection levels. The only significant interferences would be from compounds very similar to the agents, such as pesticides and herbicides.

6.1.4 Fourier-Transform Infrared (FT/IR)

Spectroscopic techniques have higher information content than other methods of detection. The potential exists for the identification of many chemicals. The difficulty in the application of spectroscopic sensing to continuous monitoring is to obtain and process all of the information available in a short period of time. This requires a fast scanning instrument combined with a computer that both operates the instrument and identifies the compounds detected. Since the spectrum will be a composite of several species, some chemometric algorithm must be applied to deconvolute the spectra. The alternative is to find a unique spectral attribute that is indicative of the chemicals of interest. For example, nerve agents have a phosphorus-oxygen double bond (Figure 7) with a characteristic frequency. By monitoring this vibrational frequency, nerve agents could be detected. There are a couple of problems with this approach. The first problem stems from the narrow linewidths for vibrations in the gas phase. Although the functional group vibrational frequencies are close (within a 100 cm^{-1}), the linewidths may be as narrow as 1.0 cm^{-1} and the IR source linewidth must be matched to include the entire band of interest. The second problem stems from other functional groups' vibrational frequencies overlapping the band. Thus, for less ambiguous detection, a multiband approach is needed. This brings

things back to the complete spectrum approach. The third problem is that some very similar compounds are common in the environment, such as the organophosphorus-based insecticides (Figure 8). For example, dichlorvos, the pesticide used in flea collars, is extremely similar in structure and atom-content to the nerve agents. Using a gas chromatograph with FT/IR detection is currently a laboratory option only. Because FT/IR detectors are intrinsically slow, scanning speed is restricted and, in order to obtain several spectra per chromatographic peak, fast GC techniques are excluded.

The IR region of the spectrum is frequently segregated into three regions. The near IR, from $0.8\text{ }\mu$ to $2.5\text{ }\mu$ ($12\,500$ to 4000 cm^{-1}) is little used. The bands in this region are generally overtones and combination bands of light atom vibrations. The most notable use of this region is for Raman spectroscopy of substances to avoid fluorescent backgrounds

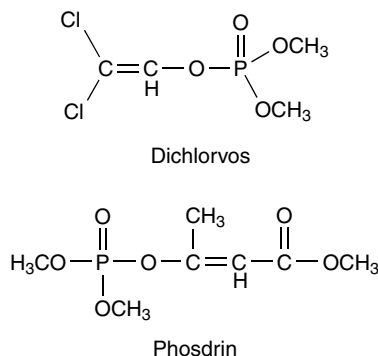


Figure 8. Chemical structures of two common insecticides

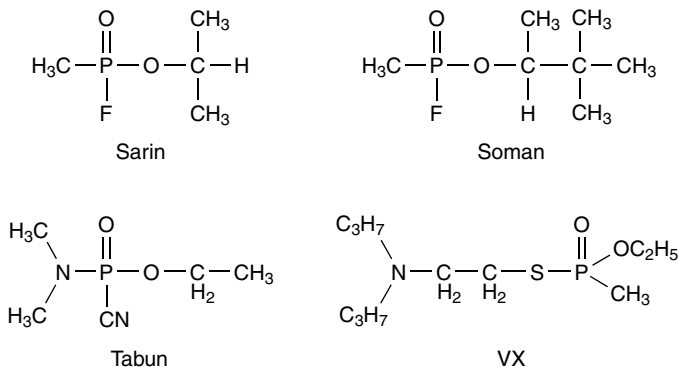


Figure 7. Chemical structures of nerve agents

using near-IR (NIR) laser excitation (Nd-YAG) and Fourier-transform spectroscopy (FTS) detection. Raman spectroscopy is another vibrational spectroscopy technique used to measure molecular vibrations. The next region of the IR spectrum is the mid-IR, which ranges from 2.5μ to 50μ (4000 to 200 cm^{-1}) and is regarded as the primary IR region. This region is divided into two subregions, the group frequency region (2.5 to 8μ) and the fingerprint region (8 to 50μ). The group frequency region is used to observe functional groups and the fingerprint region is used in spectral search methods for compound identification. Analogous to the chromophores in UV-visible absorption spectroscopy, functional groups of molecules give characteristic IR absorbances. These bands vary slightly in position due to the attached substituents (the rest of the molecule) but the analysis of functional groups is an important part of IR qualitative analysis. Almost all of the IR qualitative analysis is performed in this region. This is the region of the IR spectrum that would be used for chemical agent detection. The last region of this part of the electromagnetic spectrum is the far IR, from 50μ to 1000μ (200 to 10 cm^{-1}). This region is used to examine vibrations from heavy atoms and to observe the low energy vibrations of crystal lattices. Owing to the difficulties of detecting such small thermal energies, this region is more commonly examined by Raman spectroscopy.

So, as with the other detection strategies, interferences will be encountered from chemically similar materials. However, if the sophistication of the system is high enough, the correlations of the fingerprint region can discriminate similar compounds. The difficulties encountered are the resulting size and the expense of the detection system. Some developing systems may mitigate these issues but there are currently no commercially available monitors with this sophistication. An additional benefit of this IR spectral approach would be potentially useful detection of biological agents.

6.1.5 Mass Spectrometry

MS has long been used for the detection of low molecular weight gases. The devices are called residual gas analyzers and are typically magnetic sector or quadrupole instruments. The quadrupole mass spectrometer is usually the analyzer of choice

for gas analysis since it can tolerate higher pressures than other analyzers. The primary use for these devices is to detect molecules that survive EI ionization without extensive fragmentation. This approach can be applied to CW agent detection with the addition of detection algorithms designed to recognize the fragmentation pattern of the agents. The problem becomes the same as for IR spectra in that it is difficult to interpret spectra from a combination of compounds without prior separation. The same solutions are also often applied, monitoring for several ion masses simultaneously or using a chemical separation method like gas chromatography. Unlike FT/IR, mass spectrometers can scan rapidly, so that using the fast GC methods are applicable. Tandem MS is an approach that uses a first mass spectrometer to select a specific ion from a sample to be further fragmented and mass analyzed by a second mass spectrometer. This method could have a speed advantage but is difficult to implement when a variety of chemicals need to be detected.

6.2 Specific Chemical Interferents

6.2.1 Insecticides

OP pesticides were developed in Germany during the 1930s and 1940s. The earliest compounds were so toxic to mammals that a second group had to be developed. OP pesticides (Figure 9) readily undergo biodegradation (unlike the organochlorides they do not bioaccumulate) and are toxic to a wide range of insects. Unfortunately, some are also toxic to humans due to the similarity to chemical warfare nerve agents. One very toxic pesticide is parathion, as little as 2 mg can kill a child and several hundred children have died from exposure. Less toxic is Malathion ($\text{LD}_{50} = 1375\text{ mg/kg}$), which is hydrolyzable by carboxylase enzymes. Mammals have these enzymes, insects do not. However, these enzymes can be inhibited by other organophosphates. In the human body, these pesticides are converted to paraxon, which is an acetylcholinesterase inhibitor. Some commonly used pesticides (e.g. the organophosphate (OP) Malathion and the carbamate Sevin) and some common therapeutic drugs (the carbamates pyridostigmine [Mestinon] and physostigmine [Antilirium]) also inhibit acetylcholinesterase and

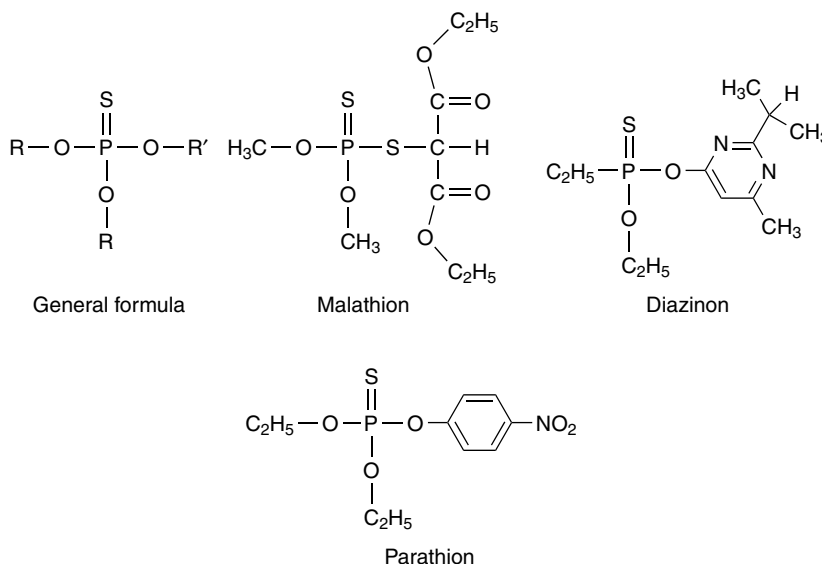


Figure 9. Some additional organophosphorous pesticides

can be considered ‘nerve agents.’ While the OP pesticides cause the same biological effects as nerve agents, there are some important differences in the duration of biological activity and response to therapy. This is due to the phosphorous-sulfur functionality instead of the phosphorous oxygen or phosphorous fluorine function of the chemical warfare nerve agents.

6.2.2 Herbicides

Glyphosate (Roundup) is a member of the organophosphate herbicides. These are nonnitrogen based herbicides that inhibit synthesis of essential amino acids and promote destruction of photosynthetic pigments in foliage. Glyphosate is a colorless to white, odorless crystal or powdery solid that is applied from the air or ground. Glyphosate is a non-selective, post emergence, broad spectrum herbicide used to control annual and perennial grasses, sedges, broadleaf, and emergent aquatic weeds. Glyphosate also serves as an insecticide for fruit tree insects. Its similarity to nerve agents can be seen by its structure (Figure 10).

Carbamates are a class of herbicides that inhibit seedling growth (Figure 11). The degradation of the nerve agent VX results in carbamates.

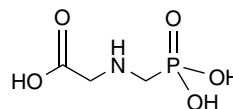


Figure 10. Glyphosate (Roundup)

Carbamates also include pesticides such as Sevin, aldicarb, and carbaryl. They are more degradable than organophosphates and have lower dermal toxicities. Their toxicity is also due to inhibition of acetylcholinesterase but they do not penetrate the central nervous system, so most effects are respiratory in nature. An acetylcholinesterase, which has been carbamylated can undergo spontaneous hydrolysis *in vivo*, which reactivates the enzyme leading to less severe symptoms or a shorter duration of symptoms. Carbaryl has a low toxicity for mammals; however, Permethrin is highly toxic to mammals, but not readily absorbed through the skin.

6.3 Summary

As discussed above, the most likely interferences come from compounds that chemically resemble CW agents. The compounds that are most like chemical warfare agents are insecticides and herbicides.

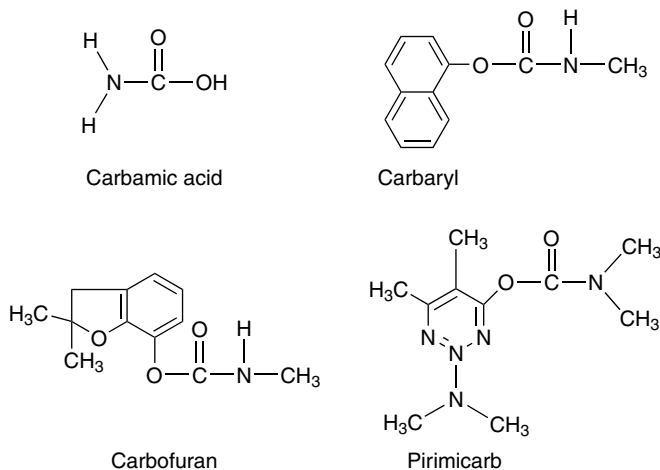


Figure 11. Carbamate herbicides

The difficulty in selecting against these interferents stems from the fact that some pesticides could be used as terrorist weapons. Therefore, the issue of selectivity is of prime importance. All of the sensor detection methods have susceptibility to interferences. The highest degree of false-positive rejection occurs when a method combines separation with selective detection. These methods are generally too slow to give a timely warning, or require large and expensive instruments. The best compromise may be to use two types of inexpensive point sensors that function by different physical mechanisms to give orthogonal data.

ABBREVIATIONS AND ACRONYMS

AC	Hydrogen Cyanide
ACADA	Automatic Chemical Agent Detection Alarm
ACAMS	Automatic Continuous Agent Monitoring System
AED	Atomic Emission Detectors
CAM	Chemical Agent Monitor
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CG	Phosgene
CI	Chemical Ionization
CK	Cyanogens Chloride
CMS	Chip Measurement System

CRD	Cavity Ring Down
CW	Chemical Weapons
CX	Phosgene Oxime
DAAMS	Depot Area Air Monitoring System
DARPA	Defense Advanced Research Projects Agency
DIAL	Differential Absorption LIDAR
EC	Electron Capture
E-CAM	Enhanced Chemical Agent Monitor
ED	Emission Detectors
EI	Electron Impact
EMC	Electromagnetic Compatibility
EMP	Electromagnetic Pulse
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
FTIR	Fourier-transform Infrared
FTS	Fourier-transform Spectroscopy
GA	Tabun
GB	Sarin
GC	Gas Chromatography
GC/AED	Gas Chromatographic/Atomic Emission Detector
GC/FPD	Gas Chromatography/Flame Photometric Detector
GC/MS	Gas Chromatography/Mass Spectrometry
GD	Soman
GID	Graseby Ionics Detector
HD	Sulfur Mustard
HF	Hydrogen Fluoride
HN	Nitrogen Mustard

HPLC	High Performance Liquid Chromatography
HRIMS	High Resolution IMS/Mass Spectrometer
HT	Hadamard Transform
ICAD	Miniature Chemical Agent Detector
ICAM	Improved Chemical Agent Monitor
ICAM-APD	Improved Chemical Agent Detector-Advanced Point Detector
IMS	Ion Mobility Spectrometers
IMS/MS	Ion Mobility Spectrometers/Mass Spectrometry
IPDS	Improved Chemical Agent Point Detection System
IR	Infrared
JCAD	Joint Chemical Agent Detector
JCBAM	Joint Chemical and Biological Agent Water Monitor
JSLSCAD	Joint Service Lightweight Standoff Chemical Agent Detector
JWARN	Joint Warning and Reporting Network
LIDAR	Light Detection and Ranging
MECC	Micellar Electrokinetic Capillary Chromatography
MEMS	Micro-Electro-Mechanical Systems
MS	Mass Spectrometry
MSD	Mass Selective Detector
NAIAD	Nerve Agent Immobilized-enzyme Alarm and Detector
NATO	North Atlantic Treaty Organization
NIR	Near-IR
OP	Organophosphorous
OPCW	Organization for the Prohibition of Chemical Weapons
QCM	Quartz Crystal Microbalances
RAPID	Remote Air Pollution Infrared Detector
SALAD	Shipboard Automatic Liquid Agent Detector
SAWS	Surface Acoustic Wave Sensors
SFAI	Swept Frequency Acoustic Interferometry
SOF	Special Operations Forces
SPME	Solid Phase Micro-Extraction
SPR	Surface Plasmon Resonance
TCD	Thermal Conductivity Detector
TIC	Toxic Industrial Chemical
TOF	Time of Flight
VAC	Volts Alternating Current

VX *O*-ethyl-S-(2-isopropylaminoethyl) methylphosphonothiolate

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CHAPTER 6

A Comprehensive Review of the Official OPCW Proficiency Test

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1 INTRODUCTION

The Chemical Weapons Convention (CWC) ⁽¹⁾ provides for the request for off-site sample analysis

during on-site inspections, in laboratories designated for this task.

The Technical Secretariat of the Organization for the Prohibition of Chemical Weapons

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(OPCW) provides a proficiency-testing scheme for the analysis of samples in the context of the CWC. The design of the scheme should simulate off-site sample analysis, whereas the objective of the scheme is to obtain a network of laboratories that are qualified for the aforementioned task. Successful participation in the scheme may lead to this qualification, that is, designation, provided a laboratory has obtained an accreditation for the analysis of CWC-related chemicals by an internationally recognized accreditation body.

From May 1996 to December 2002, 11 official OPCW proficiency tests have been conducted that led to designation by the Director-General of the OPCW Technical Secretariat. As a result, 13 laboratories out of the approximately 45 participants worldwide, have been designated: eight laboratories from the Western European and Others Group, three laboratories from the Eastern Europe Group, and two laboratories from the Asian Group; the designated laboratories are listed in Annex 1. At present, the African group is participating with one laboratory and the Latin American and Caribbean group has no regular participant.

This article reflects the Master of Science study on the first 10 official OPCW proficiency tests. The study has been performed with the objective to establish lessons learnt and to identify areas of improvement. The proficiency-testing scheme is described and studied step by step, in order to identify the challenges posed by the scheme, the suitability of the scheme, the strengths and weaknesses of the regular participants in the scheme, the reporting requirements necessary to provide a scientifically defensible report, and the weaknesses within the process of evaluation, scoring, and designation of the scheme. The study revealed that the application of a proper analysis and reporting strategy is essential to complete participation in the scheme successfully. Four categories of scheduled chemicals that create difficulties for the laboratories participating regularly have been identified. Three areas of weakness within the process of evaluation, scoring, and designation have been identified.

2 THE OPCW PROFICIENCY-TESTING SCHEME

The OPCW proficiency-testing scheme is set up with the objective to *simulate* off-site sample analysis in order to *select* (i.e. designate) laboratories that are capable of the following, with a high level of confidence:

1. perform trace analyses (ppm levels) of chemicals scheduled under the CWC and/or their degradation products in a wide variety of matrices;
2. provide the Technical Secretariat with a detailed report on the analysis results that:
 - (a) contains analytical proof of the presence of the chemicals reported and provides high certainty of the absence of other chemicals relevant for the implementation of the CWC; and
 - (b) does not contain information on chemicals not relevant for the implementation of the CWC.

To obtain and/or retain designation, laboratories must participate annually in the scheme, be accredited for the analysis of CWC-related chemicals by an internationally recognized accreditation body, and participation in the scheme must be successful. A sequence of three most recent scores is used as a measure to monitor a participant's trend in performance, and to decide upon its status of designation. In general, two proficiency tests are organized per year. Instead of participating as a regular participant, a laboratory may assist in either preparing test samples or in evaluating test results. However, the scoring obtained for these assisting tasks can only be counted toward designation once in the sequence of participation in the three most recent proficiency tests.

2.1 History

Between 1989 and 1993, the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN) initiated four international inter-laboratory comparison (round-robin) tests for the verification of chemical disarmament ⁽²⁾ to test the effectiveness of their procedures for the recovery of treaty-related chemicals (Chemical Warfare agents

and their precursors and degradation products) from various sample matrices ⁽⁴⁾. In 1994, the Provisional Technical Secretariat for the Preparatory Commission for the OPCW took over the role as coordinator of the testing scheme and subsequently organized one additional interlaboratory comparison test ⁽³⁾ to further test VERIFIN's procedures ⁽⁴⁾. From 1994 to 1995, the Provisional Technical Secretariat organized two trial proficiency tests to train laboratories and to establish procedures for the conduct of the first official OPCW proficiency test in May 1996. This test was the first test used for counting toward designation of laboratories to support the Technical Secretariat's verification activities.

2.2 Procedures

The initial procedures for the conduct of official OPCW proficiency tests have been developed by the Special Task Force on Analytical Issues in the period 1994 to 1996, and subsequently recommended for adoption through Working Group B to the Conference of States Parties; the respective procedures were adopted by the First Conference of States Parties in May 1997 ⁽⁵⁾. The evaluation criteria have been further developed since, through consultation with participants in the proficiency-testing scheme. These consultations were often initiated by practical problems posed during individual proficiency tests. The numerous amended documents have recently been compiled into three comprehensive quality system documents that describe the process of the OPCW proficiency test, which has been accredited by the Dutch accreditation body in 2001:

1. 'Standard Operating Procedure for the Organization of OPCW Proficiency Tests', QDOC/LAB/SOP/PRO001 ⁽⁶⁾;
2. 'Work Instruction for the Preparation of Test Samples for OPCW Proficiency Tests', QDOC/LAB/WI/PRO002 ⁽⁷⁾; and
3. 'Work Instruction for the Evaluation of Results of OPCW Proficiency Tests', QDOC/LAB/WI/PRO003 ⁽⁸⁾.

2.3 Process Description

The proficiency test is conducted with the assistance of two laboratories, one preparing the test samples

and one assisting in evaluating the test results. The assisting laboratories are usually OPCW-designated laboratories or laboratories that are in the process of seeking designation. Once the assisting laboratories are selected, the proficiency test is open to Member States of the OPCW that can nominate laboratories for participation. The OPCW Laboratory, which is the coordinator of the entire proficiency-testing scheme, initiates discussions with the sample preparation laboratory on the composition of the test samples and the test scenario. Subsequently, the sample preparation laboratory performs prestability studies over a longer period of time, to observe the behavior of the selected spiking chemicals in the selected sample matrices. On the basis of the prestability study results, the final sample composition is agreed upon, and the test can begin.

In the presence of an OPCW Laboratory representative, the sample preparation laboratory prepares the test samples and blanks, packs, and eventually dispatches a set to each participant in the test, including one to the evaluating laboratory and to the OPCW Laboratory. On receipt of the samples, the participants have two weeks to analyze the samples and to report their results to the OPCW Laboratory. In the same period, both the assisting laboratories and the OPCW Laboratory perform an analysis on the samples too; these analyses are performed to confirm uniformity of the sample composition and to identify any impurities and degradation/reaction-products in the test samples that could be reported by the participants, in addition to the selected spiking chemicals.

When the test period for all participants has expired, the spiking chemicals are announced and the participants' reports are opened for evaluation. A copy of the reports is sent to the evaluating laboratory, which will evaluate the reports independently and in parallel with the OPCW Laboratory. For the purpose of evaluation, all reported chemicals, other than the spiking chemicals, are categorized. Categorization is based on the analysis results obtained by the assisting laboratories and by the OPCW Laboratory. The participants are scored on the basis of the outcome of the evaluation.

The preliminary evaluation results are discussed between the OPCW Laboratory and the evaluating laboratory before this information is sent to the participants. The participants are invited to a meeting to discuss the preliminary results and to possibly

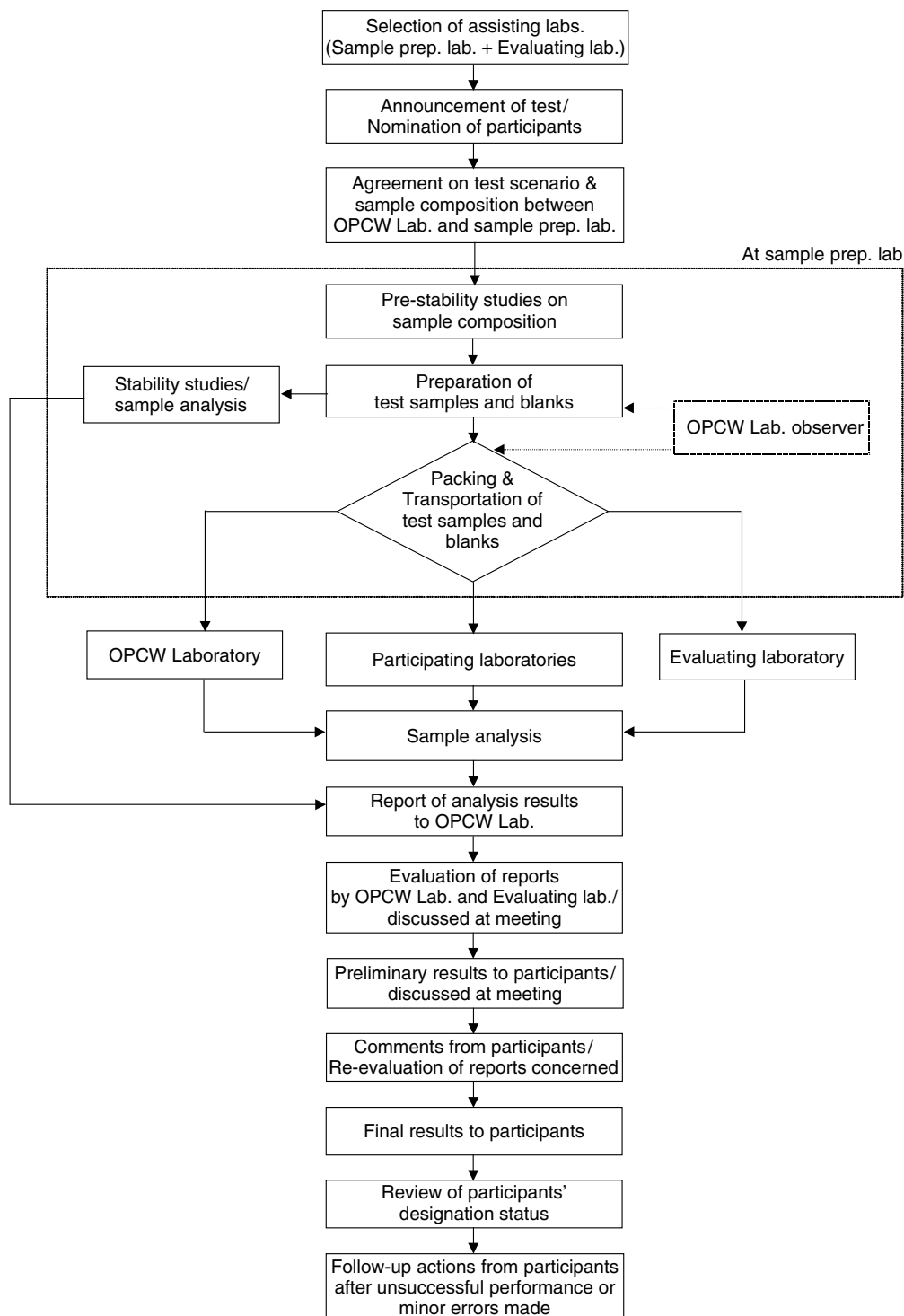


Figure 1. Process of the OPCW proficiency-testing scheme

Table 1. Schedule of OPCW proficiency-testing scheme

Proficiency test number	Sample dispatch date
1	1996, May 22
2	1996, Oct. 14
3	1997, April 22
4	1998, May 19
5	1998, Dec. 3
6	1999, Sept. 1
7	2000, March 1
8	2000, Nov. 8
9	2001, April 2
10	2001, Nov. 5

submit comments. All comments are considered for reevaluation and therefore forwarded to the evaluating laboratory for action. The final evaluation results are compiled in a final report and submitted to all participants in the test. On the basis of the final evaluation results, the participants' designated statuses are reviewed and updated accordingly. Participants who performed unsuccessfully or made (minor) errors in the test need to submit satisfactory follow-up actions before participation in the next proficiency test is sustained.

The process of the official OPCW proficiency test is shown in Figure 1.

2.4 Schedule

The schedule of the first 10 official OPCW proficiency tests is presented in Table 1.

3 THE CHALLENGES POSED BY THE SCHEME

The design of the OPCW proficiency-testing scheme is characterized as follows:

- The proficiency test involves qualitative analysis; qualitative analyses are sufficient to confirm the presence or prove the absence of CWC-related agents.
- The proficiency test involves blind testing; the participants know it is a test sample but do not know the sample composition.
- The proficiency test does not specify the analytical methods; the participants may use the methods of their choice, as long as the identifications are based on at least two independent analytical techniques leading to a consistent result.
- The proficiency test requires detailed reporting of the analysis results; the reporting requirements are specified in the quality system document QDOC/LAB/WI/PRO003⁽⁸⁾, made available to all test participants.
- The proficiency test timeline is 15 calendar days upon receipt of the samples; the test period is based on Paragraph 62 of Part II of the Verification Annex of the CWC, stipulating that the final inspection report must be completed within 30 days after the inspection.

Participation in the scheme combines sample preparation and analysis *and* detailed reporting of results. The application of an 'analysis and reporting strategy' is essential, in the attempt to complete participation successfully, within the given timeline. In the following sections, the challenges posed by the scheme will be discussed, a model for an analysis and reporting strategy will be presented, and an example of an analysis strategy and an effective sample cleanup method will be described.

3.1 Analytical Challenge

In the proficiency test, usually three different sample matrices are sent for analysis. Participants are requested to identify *any* chemical contained in the schedules of the CWC plus all the degradation products that can originate from them; this involves millions of possible chemicals (see Table 2 in **Chapter 2**). The spiking chemicals are present in low ppm levels. In most cases, additional background material(s) are added to the samples in an attempt to approach a realistic scenario; a laboratory is more likely to receive 'dirty' samples from an inspection site than 'clean' ones. The sample composition as a whole, that is, matrix, background, and spiking chemicals, differs per test. A different sample composition keeps the participants alert and simultaneously requires flexibility and creativity in preparing the samples and subsequently performing

the analysis. The analysis often requires adaptation of existing procedures.

Matrices most used were soil, water, and organic liquids. Other matrices used were, for example, a polymer strip in the first proficiency test, a metal strip covered with a thin layer of paint in the third proficiency test, and a wipe sample in the fourth proficiency test. In the ninth and tenth proficiency tests, an emulsion sample and decontamination solutions were introduced. Not all sample matrices employed in the past are considered realistic. During the Preparatory Commission of the OPCW, the Task Force on Analytical Issues (see Fourth Report of the Expert Group on Inspection Procedures, PC-VIII/B/WP.12, dated September 20, 1994) discussed that sample matrices such as wipe, rubber, paint, and concrete will be extracted on site and sent as extracts off site for analysis, after splitting.

The presence of background materials often increases the difficulty level for analysis, because the background may hide the spiking chemicals or may even react with the spiking chemicals. In fact, background is one of the main reasons why analytical data is rejected in the evaluation of proficiency tests, either because of bad quality, or because of the 'peak-in-the-blank' issue. In the worst-case scenario, the background may even result in false negative identification. The employment of an effective cleanup method is recommendable. Unfortunately, such a method is often not available and may require some method development during the test. However, within the 15-calendar-days' test period, time for 'trial and error' may not be available. For that reason, the employment of a cleanup method need not always be a participant's first choice. Background materials most used were diesel oil, polyethylene glycols, salts, and/or chemicals with similar chemical properties as the spiking chemicals applied in the same sample matrix. An example of an effective cleanup method is presented in Section 3.5.

The concentration range of the spiking chemicals is specified (ppm range, 1–10 ppm or higher ⁽⁷⁾) in order to guarantee a proper conduct of the scheme. Degradation can and may occur as long as it does not exceed 30 % of the spiking level, and as long as the concentration remains above the minimum concentration of 1 ppm ⁽⁷⁾. Usually, a spiking level of ≈ 10 ppm is applied.

3.2 Reporting Challenge

The participants' reports must include information on:

- the identified chemicals with sufficient structure information, including, at least, structural formula, CAS (Chemical Abstracts Service), registry number (if available), and chemical name (preferably IUPAC (International Union for Pure and Applied Chemistry)) or CA (Chemical Abstract) name, or the name from which the structure can be derived), and the CWC Schedule number (if available);
- the sample preparation methods employed for the recovery of the identified spiking chemicals;
- the analytical methods employed for the identification and confirmation of the identity of the spiking chemicals; and
- the analytical data supporting the identifications (chromatographic and spectrometric data from at least two different techniques).

Reporting templates are provided to accommodate the participants in this time-consuming task, and to obtain similar report structures (see Figure 2) from the different participants to simplify the evaluation.

In real off-site sample analysis, the designated laboratory's report must stand up to international scrutiny and must be legally and scientifically defensible. Within the report, an unbroken chain of evidence must therefore be established linking each sample aliquot to each chemical identified by a defined method of analysis. A way of establishing such a clear link is by naming the aliquot in the sample preparation flowchart, and subsequently using this aliquot name either as a file name or as a sample name in the instrumental analysis to be employed.

Only chemicals that are considered relevant within the scenario of the proficiency test are to be reported; to avoid irrelevant chemicals being reported, in real off-site sample analysis, confidential information on the facility under inspection is revealed (e.g. information on an industrial production process that is not relevant to the implementation of the CWC). This requirement is a consequence of Paragraph C.17 of the Confidentiality Annex of the CWC ⁽¹⁾. The reporting of irrelevant chemicals is penalized with immediate failure of the test; see Section 6.1.

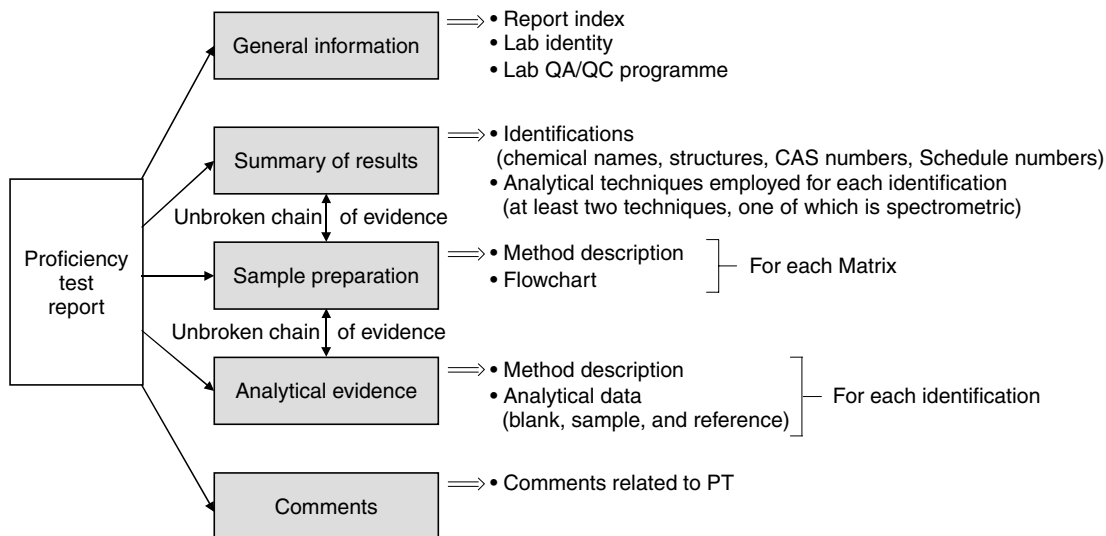


Figure 2. OPCW proficiency test report structure

The definition for the relevancy of chemicals is clear: Irrelevant chemicals are chemicals other than Scheduled chemicals or degradation products of Scheduled chemicals, or chemicals present in both sample and blank, or chemicals only present in the blank ⁽⁸⁾. The interpretation of this definition has, however, led to confusion among some of the participants in the past. The cause for confusion is demonstrated with the reporting of triethyl phosphate in the tenth proficiency test. Triethyl phosphate was present in the sample and absent in the blank. Triethyl phosphate is not Scheduled but could be an oxidation product of the Scheduled compound triethyl phosphite (Scheduled 3.B.09), and was therefore considered relevant to report. However, in making a decision on the relevancy of chemicals, participants must also take the nature of the chemicals and the test scenario into account. In general, phosphites do not easily oxidize; no traces of triethyl phosphite were present in any of the samples supporting that oxidation could have taken place. In addition, phosphates are rather common chemicals in phosphorus plants, not necessarily pointers for the presence of Scheduled phosphites. For these reasons, triethyl phosphate was categorized as being irrelevant. However, the reporting of a common chemical does not really reveal confidential information on the facility under inspection. Hence, should this reporting then lead to immediate failure of the test?

Also in the ninth proficiency test, an unscheduled oxidation product (thiodiglycol sulfoxide) of a Scheduled compound (thiodiglycol, Schedule 2.B.13) was present in the sample and absent in the blank. Again, the original Scheduled compound was not present in any of the samples in this test. However, this particular chemical *was* relevant to report; in fact, thiodiglycol sulfoxide was one of the spiking chemicals in this test. Unlike phosphites, thiodiglycol *does* easily oxidize, thus full oxidation could have taken place. In addition, thiodiglycol sulfoxide *is* a clear pointer for the presence of thiodiglycol, which in turn is a precursor for mustard gas (Schedule 1.A.04).

At present, the decision on the relevancy of chemicals is left to the participants, whereas the Technical Secretariat would be in a better position to make such judgments. After all, the Technical Secretariat has full information on the facility under inspection, whereas the participants do not receive this information.

3.3 Analysis and Reporting Strategy

The ‘analysis’ and the ‘reporting’ are the two core steps of participation in the scheme. In the attempt to identify all spiking chemicals in the analysis step, and to report them with acceptable analytical

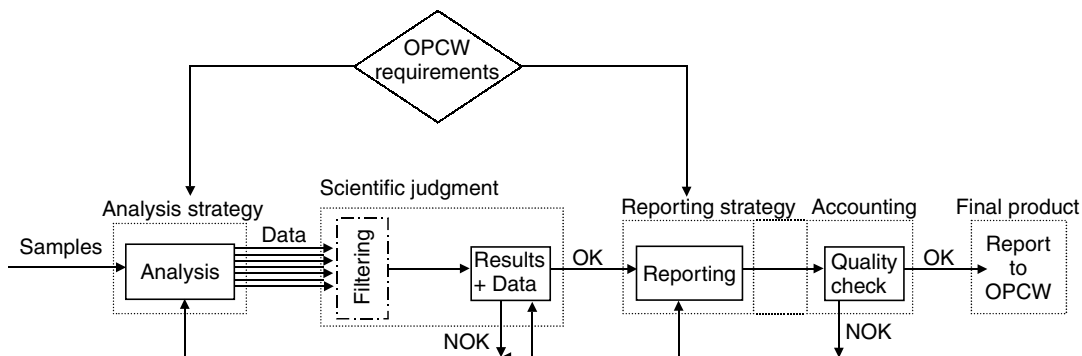


Figure 3. Model for proficiency test analysis and reporting strategy

evidence in the reporting step, it is recommendable to have a strategy in place. The involvement of various levels (e.g. laboratory staff, management, and others) within the structure of a participant's organization is necessary. Scientists should not only focus on the analysis part, but also must from the start, keep the final goal in mind, the report to the OPCW.

A model for an analysis and reporting strategy is illustrated in Figure 3:

The laboratory staff, that is, sample preparers, instrument operators, scientists, and synthesists, must join forces to analyze the samples, in order to identify the spiking chemicals with sufficient analytical evidence of acceptable/good quality. The work involves sample preparation, screening and analysis, data evaluation, and eventually making identification; an example of an *analysis strategy* is described in Section 3.4.

The data produced during the instrumental analysis must be filtered to obtain only data that (a) prove the identity of a spiking chemical, (b) are of good quality, and (c) meet the OPCW reporting requirements for the respective instrumental analysis (see reference 8), for inclusion in the final report. The initial filtering of data is performed by the instrument operators, checked by responsible scientists, and double-checked by the compiler of the report, in the *scientific judgment* step.

The report drafting should not be kept for last; it is rather time consuming due to the detailed reporting requirements that have to be met. Under time pressure, an error may easily creep in and subsequently lower the participant's final score. Reporting errors may be avoided by implementing a

reporting strategy, that is, compilation of the report on a continuous basis throughout the proficiency test period. The general part of the report, covering information on the laboratory itself and its Quality Assurance/Quality Control program, can be drafted before the test begins. The analytical part, covering the sample preparation flow-charts, the list of identifications, and the supportive analytical data, are included in the report as the analysis proceeds.

Once the report is completed, it should undergo a detailed quality check in the *accounting* step, by an independent, neutral person (e.g. Quality Assurance/Quality Control representative). The accounting is mainly to spot linking errors and/or typing errors; linking errors between relevant information/data and typing errors in, for example, chemical names, chemical structures, file names, and so on. Errors found are to be returned immediately to the staff member involved, corrected, and resubmitted for another final check. The *final product*, that is, the report, must be sent to the OPCW Laboratory for evaluation, before or on the 15th calendar day of the participant's test period.

The model for an analysis and reporting strategy presented emphasizes the importance of a structured plan of work in combination with team's commitment, in the attempt to complete participation in the OPCW proficiency-testing scheme successfully. However, teamwork among laboratory staff may not be enough; successful participation requires full support from management too. After all, management must understand that participation requires full devotion from the laboratory; other tasks preferably have to wait for a later point in time.

3.4 Example of Analysis Strategy

An analysis strategy can generally be outlined in three steps: (1) Sample preparation, (2) Screening and Analysis, and (3) Data evaluation and identification; examples are described in the following sections.

3.4.1 Sample Preparation

After arrival, checking, and coding, the proficiency test samples and corresponding matrix blanks are usually divided into portions to allow application of multiple sample preparation methods for the different analytical techniques prior to analysis ⁽⁹⁾.

Handling and preparation of authentic/proficiency test samples is preferably performed in a laboratory room dedicated to this purpose. The key advantage of a separate room is limitation of the possibilities of sample contamination. In previous tests, several participants have experienced cross-contamination, often with the consequence of false positive identifications and subsequent failure of the test.

The choice of sample preparation method strongly depends on the composition of the sample and on the requirements for the different analytical techniques employed in the screening and analysis. Because the sample composition of proficiency test samples is unknown, no target compound sample preparation methods can be applied. Hence, laboratories must use general methods to start with. Most of the laboratories use the Recommended Operating Procedures published by VERIFIN in Finland in 1994 ⁽⁴⁾, if the sample composition allows so. However, depending on the sample composition, these procedures may require modification. For example, in the ninth and tenth proficiency test decontamination solutions were introduced as 'new' sample matrices, with high concentrations of salt as background material among other chemicals. The nature of these matrices made it necessary for many participants to adapt the 'Recommended Operating Procedure for water samples', in order to recover the analytes of interest. However, this adaptation is considered realistic because laboratories must be able to modify procedures according to the requirements of the sample.

Parallel to the preparation of proficiency test samples, some laboratories do prepare quality control samples by the same methods, to check

the efficiency of the preparation methods applied. However, this is only useful when a sample preparation method is employed for the recovery of a particular class of compounds (e.g. lewisites), or when tentative identifications (e.g. from screening) have been made and the sample is subsequently cleaned up for reanalysis under different conditions. By spiking the target chemical(s) to a quality control sample, the cleanup method employed is better monitored.

3.4.2 Screening and Analysis

The first step in the analysis of CWC-related chemicals involves screening of the samples for the presence of compounds of interest. Tentative identification achieved by screening analysis may be useful to guide the method of sample preparation ⁽¹⁰⁾ and/or to find additional confirmation through the employment of other analysis techniques in obtaining unambiguous identification. It is noted that the boundary between screening and identification is not always clear-cut ⁽⁹⁾.

Initial screening of organic liquid samples focuses on the presence of volatile CWC-related chemicals. Organic liquid samples can be screened directly using techniques such as:

- Gas chromatography (GC) analysis with element-specific detectors, for example, a nitrogen–phosphorus detector (NPD), a flame photometric detector (FPD, in sulfur or phosphorus mode) and/or an atomic emission detector (AED);
- GC mass spectrometry analysis in electron impact mode (GC/EIMS);
- One-dimensional nuclear magnetic resonance spectroscopy (1D-NMR).

Screening with different element-selective detectors provides information on the presence of any nitrogen-, phosphorus-, and/or sulfur-containing compounds in the sample. The advantage of this screening technique is that many Scheduled chemicals can be detected with little or no interference by the background ⁽⁹⁾. Mass Spectrometry (MS) screening focuses more on structure-specific fragment ions that are characteristic for particular compounds or groups of compounds ⁽¹⁰⁾. 1D-NMR is initially used to screen for the presence

of phosphorus-containing compounds ($^{31}\text{P}\{^1\text{H}\}$ -NMR) (terminology: experiment phosphorus-31 observation with simultaneous proton (hydrogen-1) decoupling), fluorine-containing compounds (^{19}F -NMR) (terminology: experiment fluorine-19 observation) ⁽¹¹⁾, and for the presence of 2-chloroethyl moieties or 2-chlorovinyl moieties (^1H -NMR) (terminology: experiment proton (hydrogen-1) observation) that might originate from mustard agents or lewisites, respectively. Although little sample preparation (liquid samples can be run as such or the liquid is first partially or totally changed to a deuterated solvent) is required for NMR, background materials may interfere in the analysis ⁽¹¹⁾. Background materials, in particular, may cause problems in the ^1H -NMR analysis.

Organic liquid samples are also screened for the presence of polar nonvolatile CWC-related chemicals. The presence of such compounds cannot simply be discarded, because organic liquids may contain traces of water. GC analysis of polar nonvolatile chemicals is possible after derivatization (e.g. silylation with *N,O*-bis(trimethylsilyl) trifluoro-acetamide ⁽¹²⁾ and/or methylation with diazomethane).

GC analysis with element selective detectors and GC/MS analysis is usually performed with the same sample aliquot and under similar chromatographic conditions, for example, with the same type of stationary phase. This is done to achieve accurately comparable retention times on both instruments. This comparison in particular may prove its value when background materials interfere in a GC/MS run and complicate interpretation of the total ion chromatogram (TIC).

Initial screening of water samples and extracts mainly focuses on the presence of polar nonvolatile alkylphosphonic acids and nonvolatile breakdown products of nerve agents in Schedule 2 (Precursors Schedule 2.B.04, 2.B.11, 2.B.12, and 2.B.14) ⁽¹⁰⁾. Water samples and extracts are preferably screened without sample preparation, typically with:

- Capillary electrophoresis with flame photometric detection (CE/FPD) ⁽¹⁰⁾.
- Flow injection analysis (FIA) with electrospray ionization (ESI) or atmospheric pressure chemical ionization mass spectrometry detection (FIA/ESIMS or FIA/APCIMS) ⁽¹⁰⁾, followed by

column injection analysis on the same instrument (LC/ESIMS or LC/APCIMS).

- 1D-NMR.

Direct analysis of water samples and extracts has the advantage that no time-consuming sample preparation is required, as it would be required for the indirect GC analysis. This precludes identification of artifacts incurred during evaporation, derivatization, or by hot injection ports, which could result in false identifications.

3.4.3 Data Evaluation and Identification

The second step in the analysis of CWC-related chemicals involves unambiguous identification of target chemicals that have tentatively been identified during the screening process. Unambiguous identification of CWC-related chemicals is achieved when, at least, two different techniques give consistent results; at least one of the identification techniques must be a spectrometric technique ⁽⁸⁾.

The main spectrometric identification techniques employed are gas chromatography/mass spectrometry (GC/MS) ⁽¹³⁾, liquid chromatography/tandem mass spectrometry (LC/MS(MS)) ⁽¹⁴⁾, nuclear magnetic resonance (NMR) ⁽¹¹⁾, and/or gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR) ⁽¹⁵⁾. Each of these spectrometric techniques provides a spectrum that is characteristic of a chemical. MS and NMR spectra provide (detailed) structural information (like a 'fingerprint'), whereas an FTIR spectrum provides information on functional groups.

The proficiency test rules accept three methods of identification: (1) interpretation of spectrum, (2) comparison with a reference spectrum, and (3) comparison with a spectrum from an authentic standard. Method 1, interpretation of MS and/or NMR spectra, is often used as the first means to find out a chemical's structure. Interpretation may be considered sufficient for identification, as long as it is based on a spectrum from a closely related chemical. However, reporting interpretation, which is scientifically pure, that is, referenced to literature, may give complications within the timeline of the test, in particular when the necessary literature is not at hand. Method 2, comparison with a chemical's library spectrum, is probably a

faster method to achieve identification provided a reference spectrum is available, with a good match observed. However, Method 3, analyzing a reference standard of the suspected chemical under the same analysis conditions as the sample, is considered to be the best method of all. In the latter method, not only the spectra but also the retention times can be compared, providing double analytical evidence.

Method 3, however, poses an additional challenge to the participants if no reference standard is available because it implies that the participants must have the chemical synthesized. Laboratories that are capable of synthesizing CWC-related compounds, in general, show a high competence in the analysis of authentic/proficiency test samples. Commercially available Scheduled compounds, alcohols, and thioalcohols are listed in Annex 2.

'Retention parameter' techniques (such as GC, CE, or LC techniques equipped with selective detectors) are accepted as identification techniques, however, only when used in combination with at least one of the spectrometric techniques listed above, and when compared to the results obtained from a reference standard analyzed under similar conditions as the sample, giving consistent results.

The analysis strategy described shows what a tremendous amount of work the analysis of proficiency test samples actually holds. This example is just one approach that could possibly lead to successful completion of the analysis within the short timeline of the test. In practice, there may, of course, be other approaches leading to a similar result.

3.5 Example of Cleanup Method

Large amounts of background chemicals, present in the sample matrix, may give complications in all spectrometric analysis techniques. This holds particularly true for NMR experiments as a result of resonance overlap in the absence of a compound separation method. MS and FTIR techniques are generally used as detection methods with a separation method, but in these techniques a chromatographic peak overlap may pose a problem. One way to solve a chromatographic overlap is reanalysis of the sample with a chromatographic column of a different polarity. Another way to obtain tentative identification in case of MS is through target screening

(with an extracted ion chromatogram (EIC), instead of the TIC) and subsequent subtraction of a selected background spectrum. However, the quality of the spectrum thus obtained is often poor, as compared to the chemical's library/reference spectrum. Hence, there is no single guaranteed solution to the problem of co-elution in GC/FTIR and GC or LC/MS.

Background chemicals present in both the sample and the corresponding matrix blank may create an additional problem. The proficiency test rules require, for proof, that the analyte of interest is not present in the blank. Any peak in the blank that is eluting within the specified retention time window of the peak of interest in the sample must be explained, either by reporting the relevant spectra or, by reporting EICs of the sample and the blank. However, when many background peaks are present, spectrum reporting is an elaborate job and may reveal irrelevant information. For these reasons, EIC reporting is the most effective method, provided that the selected ion(s) from the analyte of interest are not present in the co-eluting background chemicals.

An alternative to the various analytical solutions mentioned above is the employment of a cleanup method that 'removes' the background chemicals from the sample matrix without 'loosing' the analyte(s) of interest; an example is presented in Section 3.5.1. However, before a sample can be cleaned effectively the participants must have an idea of the sample composition. And even then, the participant may not have the time to develop an effective cleanup method in its 15-calendar-days test period. For that reason, a cleanup method need not always be a participant's first choice.

3.5.1 Removal of Hydrocarbons from Organic Matrix

One of the samples in the eighth proficiency test was an organic liquid sample with dichloromethane (CH_2Cl_2) as solvent. The sample was spiked with:

- Spiking chemicals:
 - *O,O*-Dipropyl *N*-methyl-*N*-isopropylphosphoramidate (10 ppm)
 - *S*-Ethyl *O*-3-methylbutyl methylphosphonothiolate (10 ppm)

- *O,O*-Diisopropyl *N*-methyl-*N*-propylphosphoramidate (10 ppm)
- *S*-Butyl *O*-ethyl isopropylphosphonothiolate (10 ppm)

- Background material: Diesel (500 ppm)

3.5.2 Screening and Analysis

Four nitrogen- and/or phosphorus-containing chemicals were tentatively identified through direct injection of the sample by GC/NPD. Subsequent confirmation was achieved by running the same aliquot by GC/EIMS, under similar chromatographic conditions and using the same stationary phase column, although hydrocarbons from diesel were present in

the GC/EIMS TIC. In this particular case, no sample cleanup was required to identify the analytes of interest in the GC/EIMS run. As a means of confirmation, GC/CIMS with a proper mode of reaction gas (ammonia) was used.

However, in many cases, a background like diesel distinctly interferes in the GC/MS analysis and subsequently complicates identification. Of course, this problem might be overcome by the application of one of the 'solutions' mentioned in the introduction of Section 3.5. However, the (rather simple) cleanup method, developed by the OPCW Laboratory, proved to be effective too, at least, for the sample composition in this particular test. This cleanup method is described in Figure 4. TICs resulting from injection of an aliquot of the organic

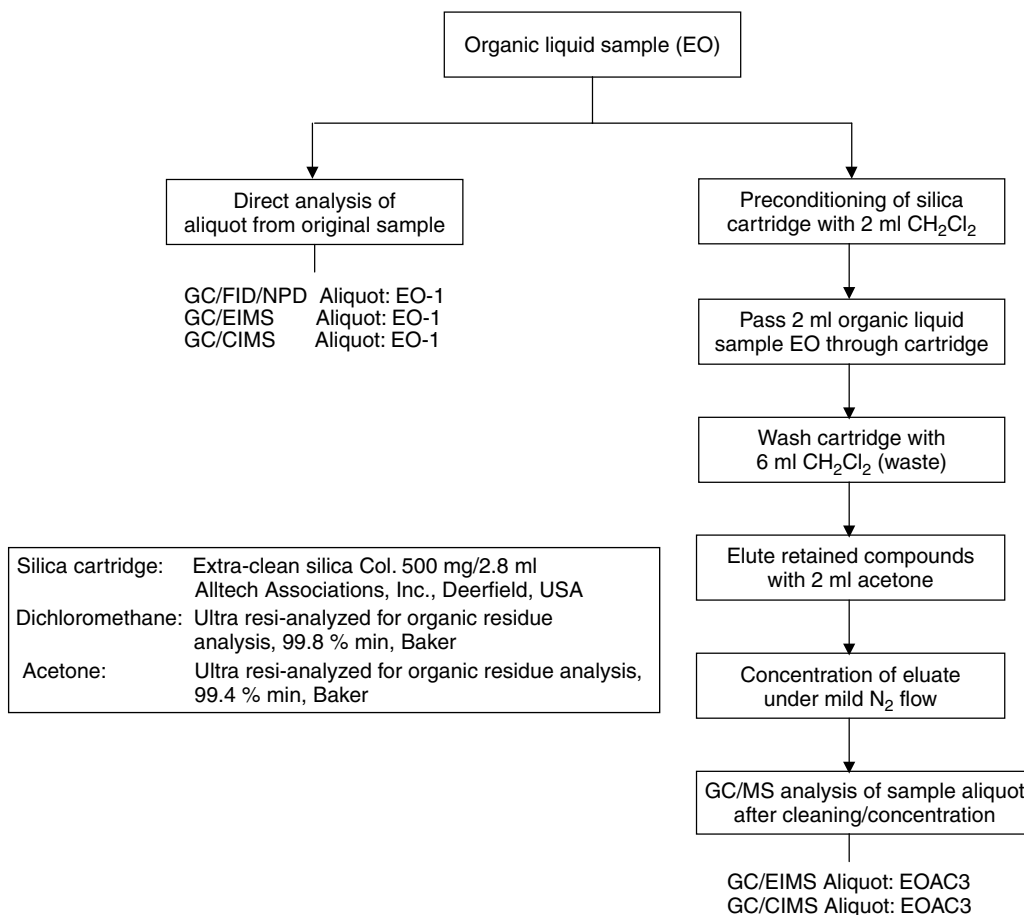


Figure 4. Sample preparation flowchart of an organic liquid sample in the eighth proficiency test

liquid sample, *before* and *after* the cleanup, are presented in Figure 5.

The efficiency of a cleanup method depends on the type of background chemicals to be removed and the type of target chemicals to be recovered. This particular cleanup method proved to be effective for the organic liquid sample composition in the eighth proficiency test.

4 TEST REVIEW

4.1 Proficiency Test versus Off-site Sample Analysis

The objective of the OPCW proficiency-testing scheme, which is to *simulate* off-site sample analysis,

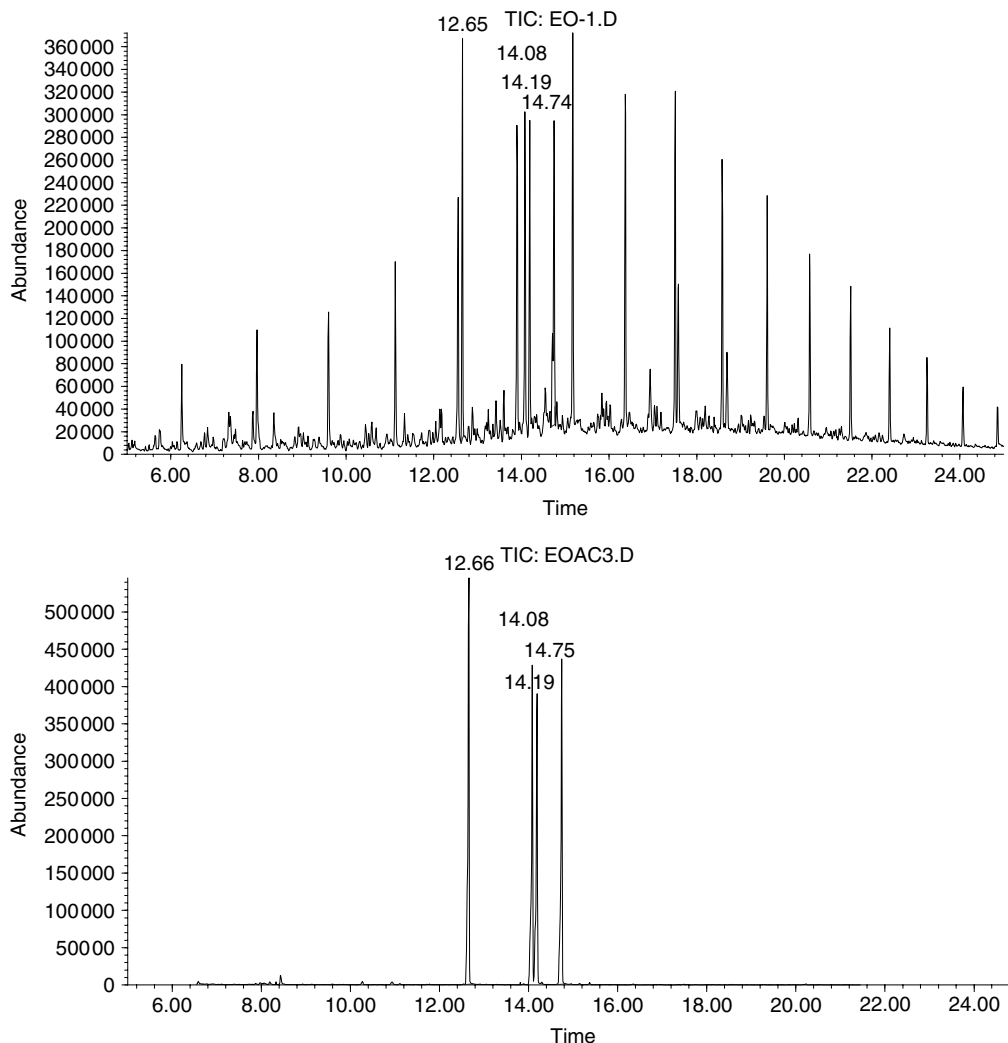


Figure 5. GC/EIMS TIC of the organic liquid (top: original sample; bottom: sample after silica cleaning, concentrated). Fused capillary column (25 m \times 0.32 mm ID, d_f 0.25 μ m) coated with CPSil8CB; carrier gas, helium at 0.9 ml/min. Temperature program: 40 $^{\circ}$ C (2 min), 10 $^{\circ}$ C/min to 280 $^{\circ}$ C (10 min). Peak designation: Rt = 12.66 min *O,O*-Dipropyl *N*-methyl-*N*-isopropylphosphoramidate, Rt = 14.08 min *S*-Ethyl *O*-3-methylbutyl methylphosphonothiolate, Rt = 14.19 min *O,O*-Diisopropyl *N*-methyl-*N*-propylphosphoramidate, Rt = 14.75 min *S*-Butyl *O*-ethyl isopropylphosphonothiolate

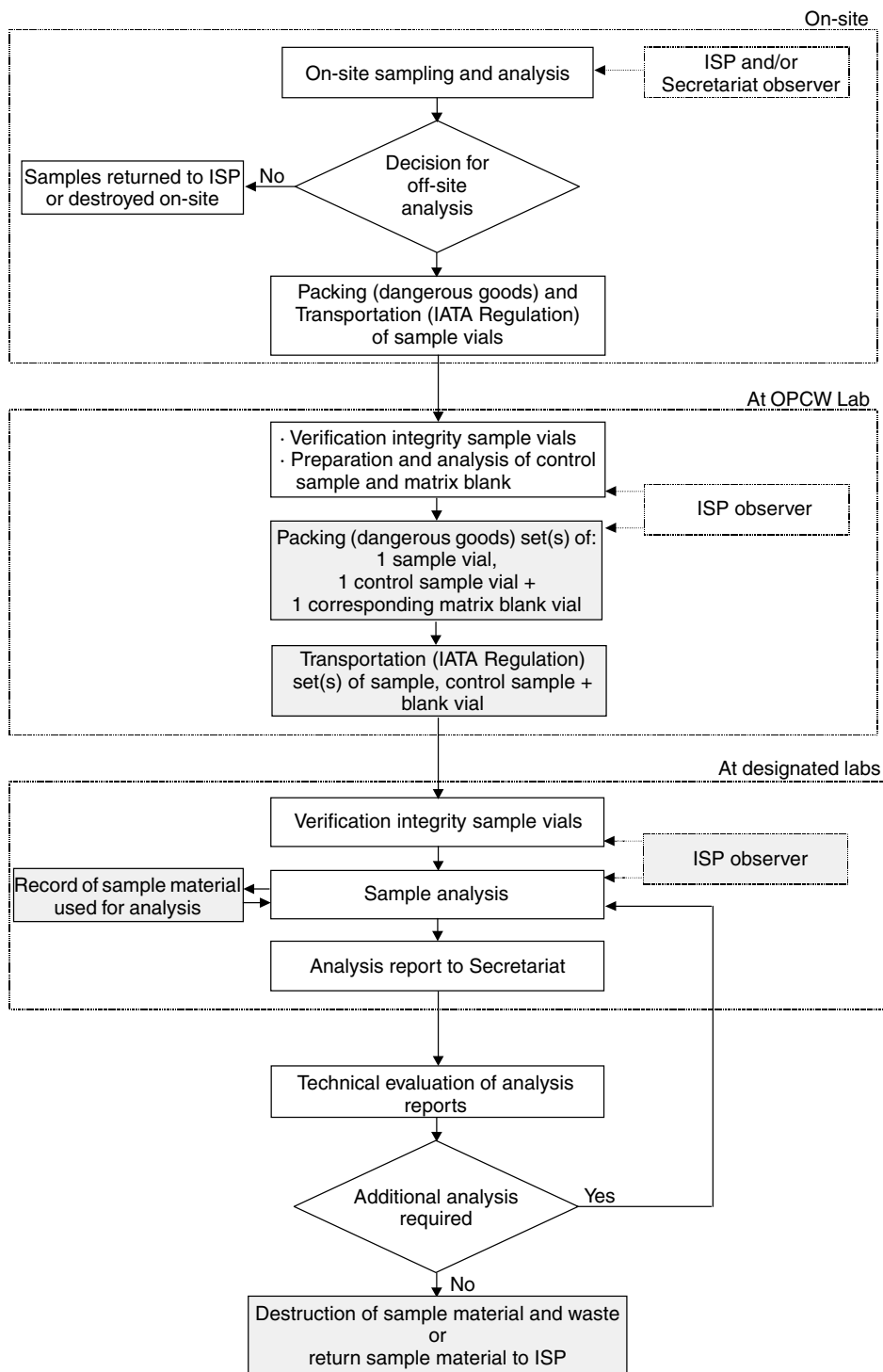


Figure 6. Process off-site sample analysis

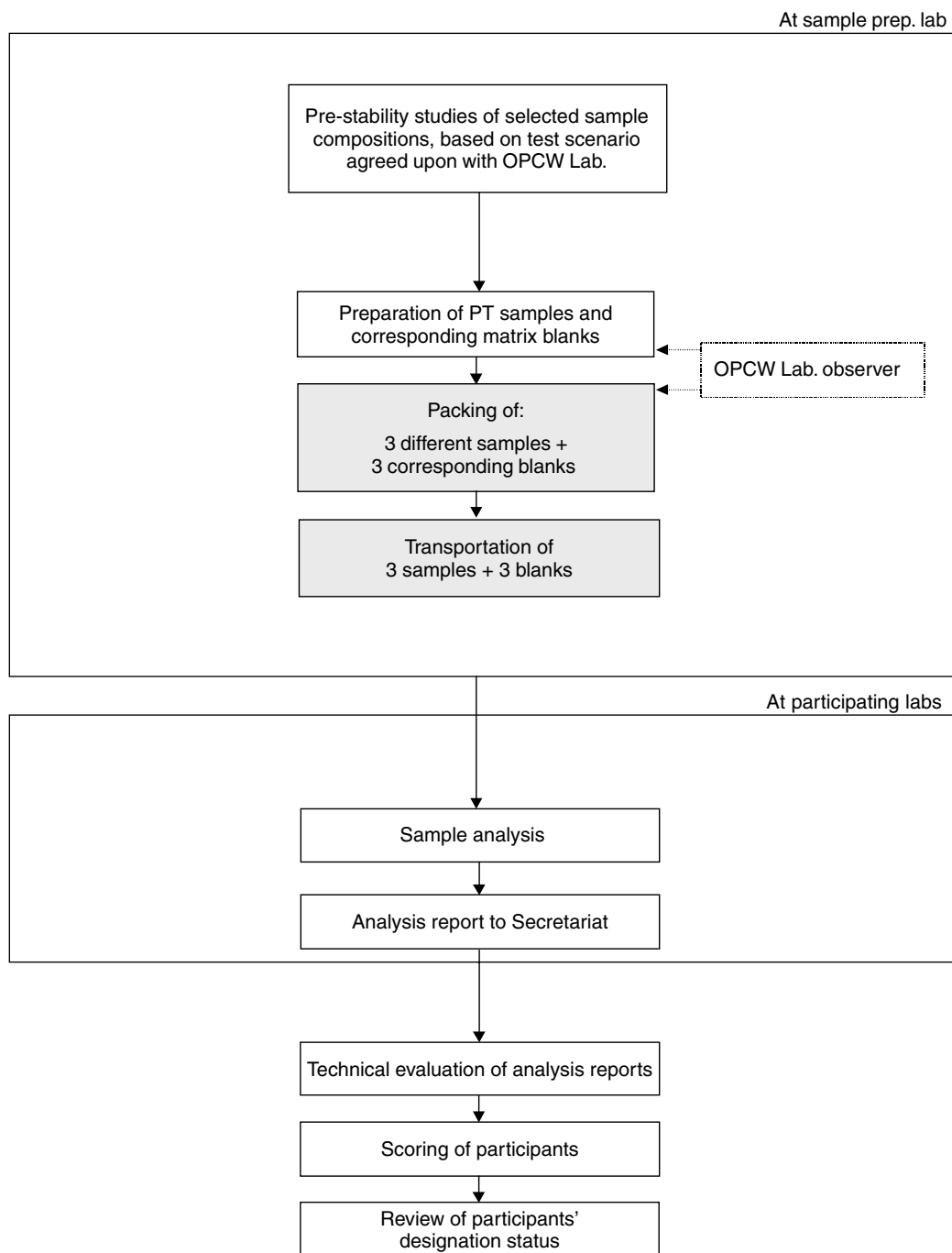


Figure 7. Simplified overview process PT

is partially met. One important element, the set of samples, still reflects the old sampling philosophy, in which it was assumed that samples and matrix blanks were both taken from the inspection site. Anticipated problems with collecting true blanks on site and unknown effects of such blank matrices on spiking chemicals when preparing control samples changed the sampling philosophy. This was done, however, without updating the process of the proficiency-testing scheme. Other elements not simulated by the proficiency-testing scheme are either not feasible to simulate or simulation is considered beyond the scope of the test. Figures 6 and 7 illustrate both processes for comparison; areas not simulated are highlighted.

4.2 Test Sample Composition

The sample composition (i.e. matrix, spiking chemicals, and background materials) applied in the first 10 official OPCW proficiency tests are summarized in Table 2. This table shows that the employment of a 'blank sample' containing no relevant chemicals has not been considered. Instead, often an artificial sample composition has been used. An example is the ninth proficiency test with the employment of (1) thiodiglycol sulfoxide (a degradation product of a blister agent) in the emulsion sample, (2) pinacolyl alcohol (a precursor of nerve agents) and Lewisite 1 and Lewisite 2 (vesicants) in the organic sample, and

(3) bis(2-diisopropylaminoethyl) disulfide (a degradation product of a nerve agent) and chloropicrin (an asphyxiating agent) in the decontamination sample. A 'cocktail' of different Scheduled chemicals within one proficiency test is not always realistic; however, it is considered unavoidable when training participants in as many different Scheduled chemicals in as few possible tests.

An overview on the number and type of *Scheduled* chemicals, used for spiking, is presented in Figures 8, 9, and 10. Approximately 21 % of the spiking chemicals belong to Schedule 1, 71 % to Schedule 2 (49 % of which belong to Schedule 2.B.04), and 8 % to Schedule 3. From Schedule 1, no nitrogen-mustards (Schedule 1.A.6) or precursors have been considered for spiking. The same applies to the degradation products (Benzilic acid (Schedule 2.B.8) and quinuclidin-3-ol (Schedule 2.B.9)) of 3-Quinuclidinyl benzilate (Schedule 2.A.3), even though the latter chemical has been used for spiking in the fourth proficiency test. Schedule 3 chemicals have been covered quite well, considering this Schedule only contains 17 different chemicals.

Test participants should be trained for all possible scenarios. In future tests, it is therefore recommendable to consider the employment of 'blank samples', as well to consider the employment of such chemicals from Schedules 1–3, which have not been considered for spiking in the past.

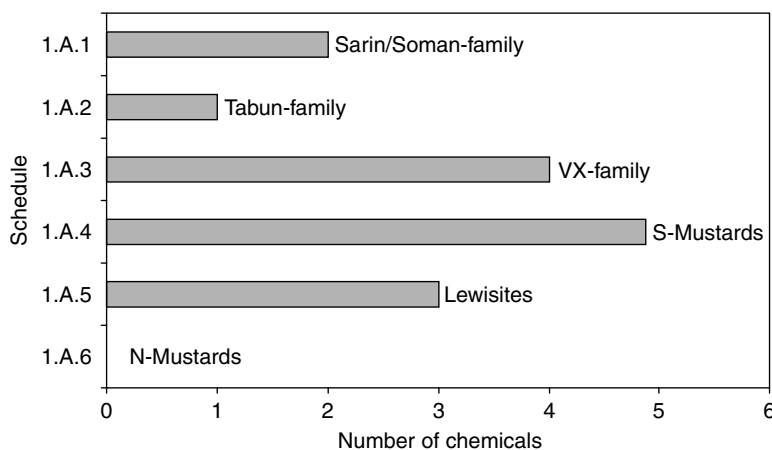


Figure 8. Number of toxic chemicals of Schedule 1 used in proficiency tests 1 to 10

Table 2. Summary of sample composition applied in proficiency test 1 to 10

Test no.	Inspection scenario	Matrix sample	Background material added to sample and blank	Spiking chemicals	Schedule
1	None	Organic (Chloro-benzene)	Diesel	<i>O</i> -Ethyl <i>N,N</i> -dimethyl phosphoramidocyanidate (Tabun)	1.A.2
				<i>N,N</i> -Dimethyl phosphoramidic dichloride	2.B.5
				<i>O,O</i> -Diethyl <i>N,N</i> -dimethyl phosphoramidate	2.B.6
		Water (lake)	Polyethyleneglycols (PEGs) + 0.1 % dichloromethane (CH ₂ Cl ₂)	Propylphosphonic acid	2.B.4
				3,3-Dimethyl-2-butanol (Pinacolyl alcohol)	2.B.14
		Polymer strip	None	1,2-Bis(2-chloroethylthio)-ethane (Sesquimustard)	1.A.4
				1,3-Bis(2-chloroethylthio)-propane	1.A.4
				1,5-Bis(2-chloroethylthio)-pentane	1.A.4
2	None	Organic (Hexane)	Diesel	<i>O</i> -Ethyl <i>S</i> -2-dimethylaminoethyl <i>n</i> -propylphosphonothiolate	1.A.3
				<i>O</i> -Ethyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate	1.A.3
		Water (underground)	PEGs + 0.1 % CH ₂ Cl ₂	Ethylphosphonic acid	2.B.4
				<i>O</i> -Isopropyl ethylphosphonic acid	2.B.4
				Triethanolamine	3.B.17
		Soil	Diesel in CH ₂ Cl ₂	<i>O</i> -Isopropyl <i>O</i> -2-methoxyethyl methylphosphonate	2.B.4
				<i>O,O</i> -Diethyl isopropylphosphonate	2.B.4
				<i>O</i> -Ethyl <i>O</i> -2-methoxyethyl isopropylphosphonate	2.B.4
3	IAU	Organic (Toluene)	Diesel	<i>O</i> -(2-Methylpropyl) <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	1.A.3
				<i>O,O</i> -Diethyl <i>S</i> -(2-(diethylamino)ethyl) phosphorothiolate (Amiton)	2.A.1
		Water (tap)	0.1 % CH ₂ Cl ₂	Isopropylphosphonic acid	2.B.4
				<i>O</i> -2-Ethylhexyl methylphosphonic acid	2.B.4
		Paint	None	<i>O</i> -2-Ethylhexyl methylphosphonofluoridate	1.A.1
				<i>O</i> -Isopropyl propylphosphonofluoridate	1.A.1
				<i>O,O</i> -Bis(2-methoxyethyl) methylphosphonate	2.B.4
4	Challenge	Water (tap)	PEGs ^a + Fatty acids ^a + 0.1 % CH ₂ Cl ₂	<i>O</i> -Propyl propylphosphonic acid	2.B.4
				<i>O</i> -Propyl propylphosphonothiolic acid	2.B.4
				<i>N,N</i> -Dipropylaminoethane-2-ol	2.B.11
		Soil	None	3-Quinuclidinyl benzilate (BZ)	2.A.3
				Bis(2-hydroxyethyl)sulfide (Thiodiglycol)	2.B.13

(continued overleaf)

Table 2. (continued)

Test no.	Inspection scenario	Matrix sample	Background material added to sample and blank	Spiking chemicals	Schedule
5	Challenge	Wipe	Diesel	<i>O,S</i> -Diethyl methylphosphonothiolate	2.B.4
				<i>O</i> -Ethyl <i>S</i> -2-ethylthioethyl methylphosphonothiolate	2.B.4
		Organic (CH ₂ Cl ₂) extract wipe	Diesel	<i>O</i> -Cyclohexyl <i>O</i> -methyl methylphosphonate	2.B.4
				<i>O</i> -Methyl <i>O</i> -pinacolyl methylphosphonate	2.B.4
		Water extract wipe	None	<i>O</i> -Pinacolyl methylphosphonic acid	2.B.4
		Water (tap)	PEGs ^a + 0.1 % CH ₂ Cl ₂	Methylphosphonic acid	2.B.4
				<i>O</i> -Cyclohexyl methylphosphonic acid	2.B.4
		Soil	Diesel in CH ₂ Cl ₂	<i>O</i> -Ethyl methylphosphonic acid	2.B.4
				<i>O</i> -Ethyl <i>O</i> -2-(1-methoxypropyl) methylphosphonate	2.B.4
6	Challenge	Organic (Dodecane)	Diesel ^a	1,5-Bis(2-chloroethylthio)-pentane	1.A.4
				2-Chlorovinyl dichloroarsine (Lewisite 1)	1.A.5
		Water (tap)	PEGs ^a + Phosphoric acid ^a + 0.1 % CH ₂ Cl ₂	Ethylphosphonic acid	2.B.4
				Ethyl diethanolamine	3.B.15
				Methyl diethanolamine	3.B.16
		Soil	Diesel ^a + Trimethyl phosphate ^a	<i>O,O</i> -Dimethyl ethylphosphonate	2.B.4
				1,5-Bis(2-hydroxyethylthio)-pentane	—
7	Challenge	Organic (CH ₂ Cl ₂)	Diesel ^a	<i>O</i> -Ethyl <i>O</i> -2-methylcyclohexyl methylphosphonate	2.B.4
				<i>O,O</i> -Dipropyl isopropylphosphonate	2.B.4
				<i>O</i> -Butyl <i>O</i> -ethyl isopropylphosphonate	2.B.4
		Water (deionized)	PEGs ^a + 0.1 % CH ₂ Cl ₂	Isopropylphosphonic acid	2.B.4
				<i>N,N</i> -Diisopropylaminoethane-2-ol	2.B.11
				Triethanolamine	3.B.17
		Soil	Diesel ^a	<i>O</i> -Propyl isopropylphosphonic acid	2.B.4
8	Challenge	Organic (CH ₂ Cl ₂)	Diesel	<i>S</i> -Ethyl <i>O</i> -3-methylbutyl methylphosphonothiolate	2.B.4
				<i>S</i> -Butyl <i>O</i> -ethyl isopropylphosphonothiolate	2.B.4
				<i>O,O</i> -Diisopropyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidate	2.B.6
				<i>O,O</i> -Dipropyl <i>N</i> -methyl- <i>N</i> -isopropylphosphoramidate	2.B.6
		Water (collector)	PEGs + 0.1 % CH ₂ Cl ₂	<i>O</i> -3-Methylbutyl methylphosphonic acid	2.B.4

Table 2. (continued)

Test no.	Inspection scenario	Matrix sample	Background material added to sample and blank	Spiking chemicals	Schedule
9	Challenge	Soil	Diesel	<i>O</i> -1,3-Dimethylbutyl methylphosphonic acid	2.B.4
				<i>O</i> -Ethyl <i>O</i> -2-ethylhexyl methylphosphonate	2.B.4
				<i>O</i> -2-Methylpentyl <i>I</i> -propyl ethylphosphonate	2.B.4
		Organic (CH ₂ Cl ₂)	Diesel + Tetrachloroethylene + Chlorobenzene + <i>p</i> -Xylene + Dodecane + (Tributylphosphate + Sulfur + Hexanol) ^b	Trichloronitromethane (Chloropicrin) Bis(2-diisopropylaminoethyl) disulfide	3.A.4 –
		Emulsion from water	Diesel + As ₂ O ₅ + <i>p</i> -Xylene + 0.5% CaCl ₂ + Butylphosphonic acid ^a	Bis(2-hydroxyethyl) sulfoxide (Thiodiglycol sulfoxide)	–
		Decon (water)	Diesel + 4% CaCl ₂ + (Octanol + Decanol + Butylphosphonic acid) ^a + (Tributylphosphate + <i>n</i> -Hexanol + As ₂ O ₅) ^b	2-Chlorovinyl dichloroarsine (Lewisite 1)	1.A.5
				Bis(2-chlorovinyl) chloroarsine (Lewisite 2)	1.A.5
				3,3-Dimethyl-2-butanol (Pinacolyl alcohol)	2.B.14
10	Challenge	Organic (Hexane)	(Chlorobenzene + <i>o</i> -Dichlorobenzene + Pyridine + Sulfur + Diethylsulfate + Bis(2-chloroethyl)ether + <i>O</i> , <i>O</i> -diethyl <i>S</i> -ethylthioethyl thiophosphate + Tris(2-chloroethyl) phosphate) ^a	<i>O</i> -Ethyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	1.A.3
				Bis(2-chloroethyl)sulfide (Mustard gas)	1.A.4
				Dimethyl phosphite	3.B.10
		Decon (deionized water)	1 % CaCl ₂ + 0.1% CH ₂ Cl ₂ + Phosphoric acid + Sulfuric acid + (Triethylamine + 2-Chlorophenol + 2, 4-Dichlorophenol) ^a	Methylphosphonic acid	2.B.4
				<i>O</i> -Ethyl <i>O</i> -2-methoxyethyl methylphosphonate	2.B.4
		Decon (deionized water pH ≈ 5)	1 % NaCl + 0.1% CH ₂ Cl ₂ + Diethylenetriamine + 2-Methoxyethanol + (Dimethyl sulfoxide + Thioanisole + Sulpoloane + Diethyl phosphate + Triethyl phosphate + Triethyl thiophosphate) ^a	Divinyl sulfoxide Bis(2-hydroxyethyl)sulfone	–

^aNot added to matrix blank sample^bNot added to sample – Degradation product of Scheduled chemical or precursor.

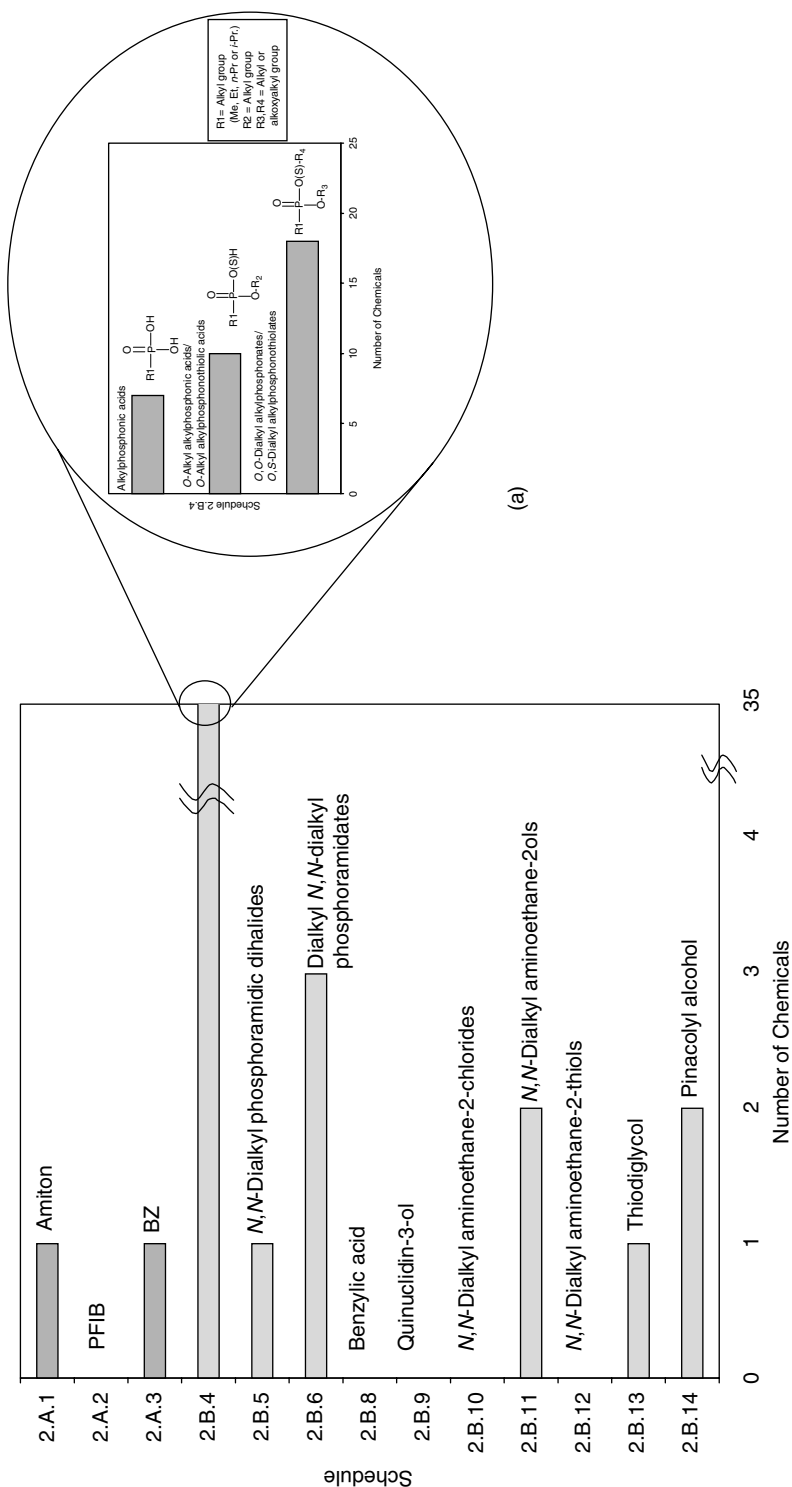


Figure 9. Number of toxic chemicals and precursors of Schedule 2 used in proficiency tests 1 to 10; Figure 9(a) Precursors of Schedule 2.B.04 used in proficiency tests 1 to 10

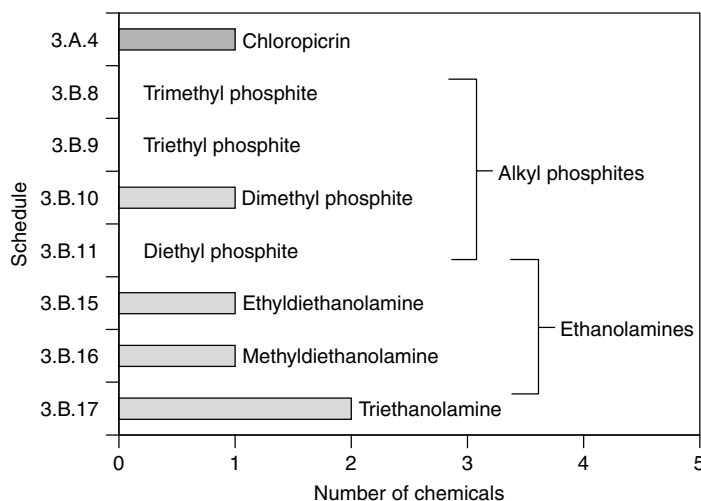


Figure 10. Number of toxic chemicals and precursors of Schedule 3 used in proficiency tests 1 to 10

4.3 Test Degree of Difficulty

Designated laboratories or laboratories seeking designation must participate in *one* of the usually *two* proficiency tests conducted annually. The proficiency tests conducted within the same year should therefore have the same degree of difficulty in order to accommodate a fair and consistent proficiency-testing scheme among the participants.

An attempt to judge the degree of difficulty of the first 10 conducted proficiency tests is made in Figures 11 and 12. The degree of difficulty for each proficiency test is measured in the percentage

of participants who *did not* manage to identify *all* the spiking chemicals; this measure does not include those laboratories that correctly identified all spiking chemicals, however, reported one or more with insufficient evidence. The participants have been split into designated laboratories (Figure 11) and nondesignated laboratories (Figure 12), respectively. The charts show that, with this measure, it can be assumed that the proficiency tests have not been similar in difficulty. The observed differences in the degree of difficulty between proficiency tests could be explained by the changing sample composition, that is, different matrices, spiking chemicals,

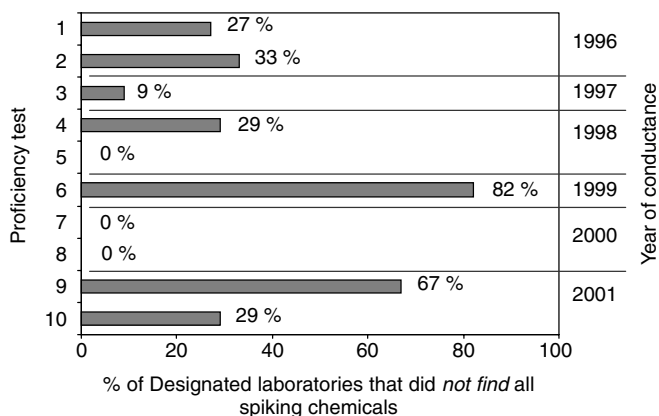


Figure 11. The degree of difficulty of proficiency tests 1 to 10, measured for the designated laboratories

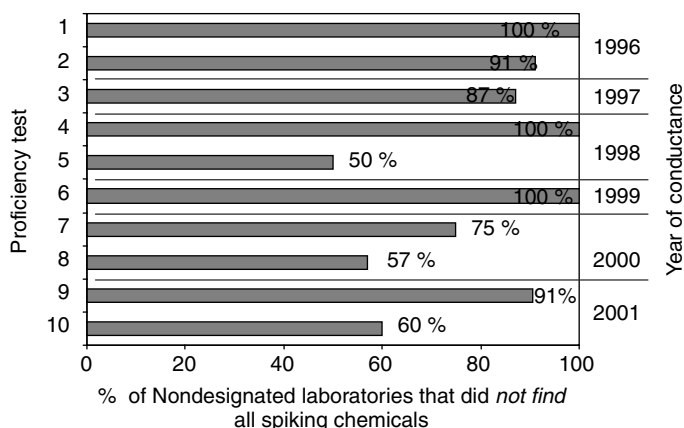


Figure 12. The degree of difficulty of proficiency tests 1 to 10, measured for the nondesignated laboratories

and background materials, and by a laboratory's degree of experience in the analysis of CWC-related chemicals.

The Technical Secretariat tries to maintain a similar degree of difficulty between proficiency tests, by controlling the sample composition for each individual test. However, not all problems that participants may face when analyzing the samples can be anticipated in advance of a test. It is a participant's responsibility to gain experience through trial and error, for example, by regular participation in the OPCW proficiency-testing scheme. Hence, the degree of difficulty of a proficiency test can only be controlled to a certain extent.

5 TEST PARTICIPANTS

The laboratories participating in the OPCW proficiency-testing scheme until December 2002 can be divided into a group of 13 designated laboratories and a group of 30 nondesignated laboratories;

see Figure 13. Within the group of nondesignated laboratories, 9 laboratories still participate regularly in the scheme. The performance of the designated laboratories and the regularly participating nondesignated laboratories will be discussed.

5.1 Overall Performance

The overall performance of the each test participant is measured in the absolute number of spiking chemicals (Figure 14). The participants are named after the State Party in which the laboratory is located. The participants are listed in alphabetical order. The three bars represent:

- Gray bar: The number of spiking chemicals correctly identified and reported;
- White bar: The number of spiking chemicals correctly identified, however reported with insufficient supportive (analytical) evidence; and
- Black bar: The number of spiking chemicals not reported.

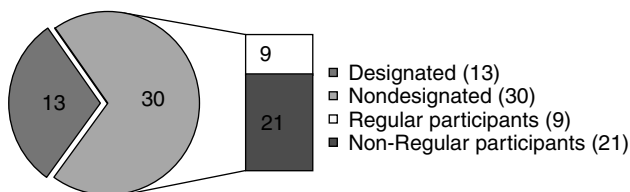


Figure 13. Laboratories participating in the OPCW proficiency-testing scheme

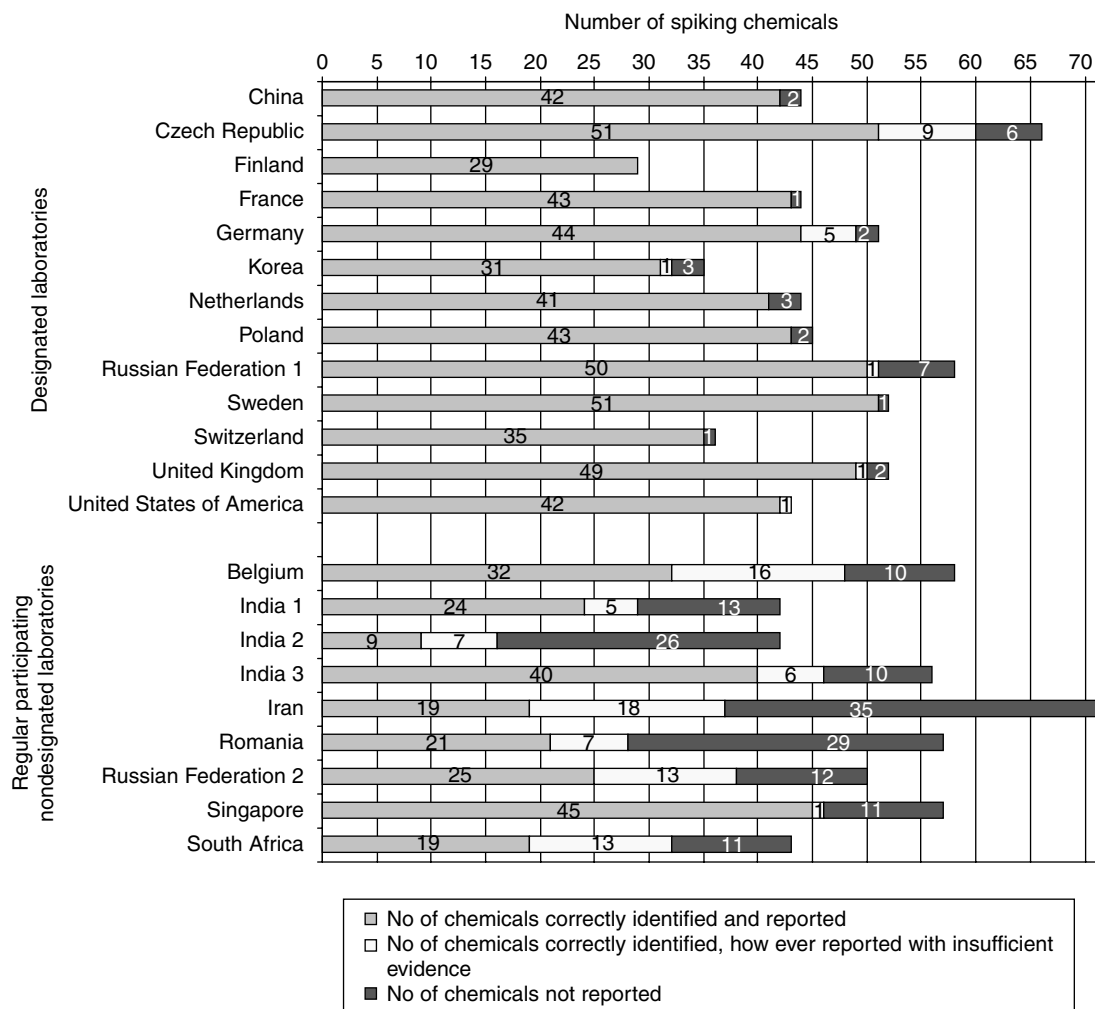


Figure 14. Overall performance of participants in proficiency tests 1 to 10, measured in the absolute number of spiking chemicals

The sum of these three bars represents the total number of spiking chemicals a participant has been tested for during the first 10 proficiency tests, with a maximum of 72. These numbers depend on the frequency of participation as regular participants, and therefore differ from the other participants. The fact that not all participants were tested on exactly the same spiking chemicals *plus* taking into account the spread in the degree of difficulty of past proficiency tests (see Section 4.3) limits the extent to which the participants' performances can be compared. The absolute numbers of

spiking chemicals in Figure 14 are translated into percentages in Figure 15. The overall performance, measured in the percentage of spiking chemicals identified and reported with or without sufficient evidence, of the designated laboratories varies from 88 to 100 %, and of the regularly participating nondesignated laboratories from 31 to 83 %. Two designated laboratories (Czech Republic and Germany) have reported a significant number of spiking chemicals with insufficient evidence; the majority of these chemicals is related to the first proficiency test.

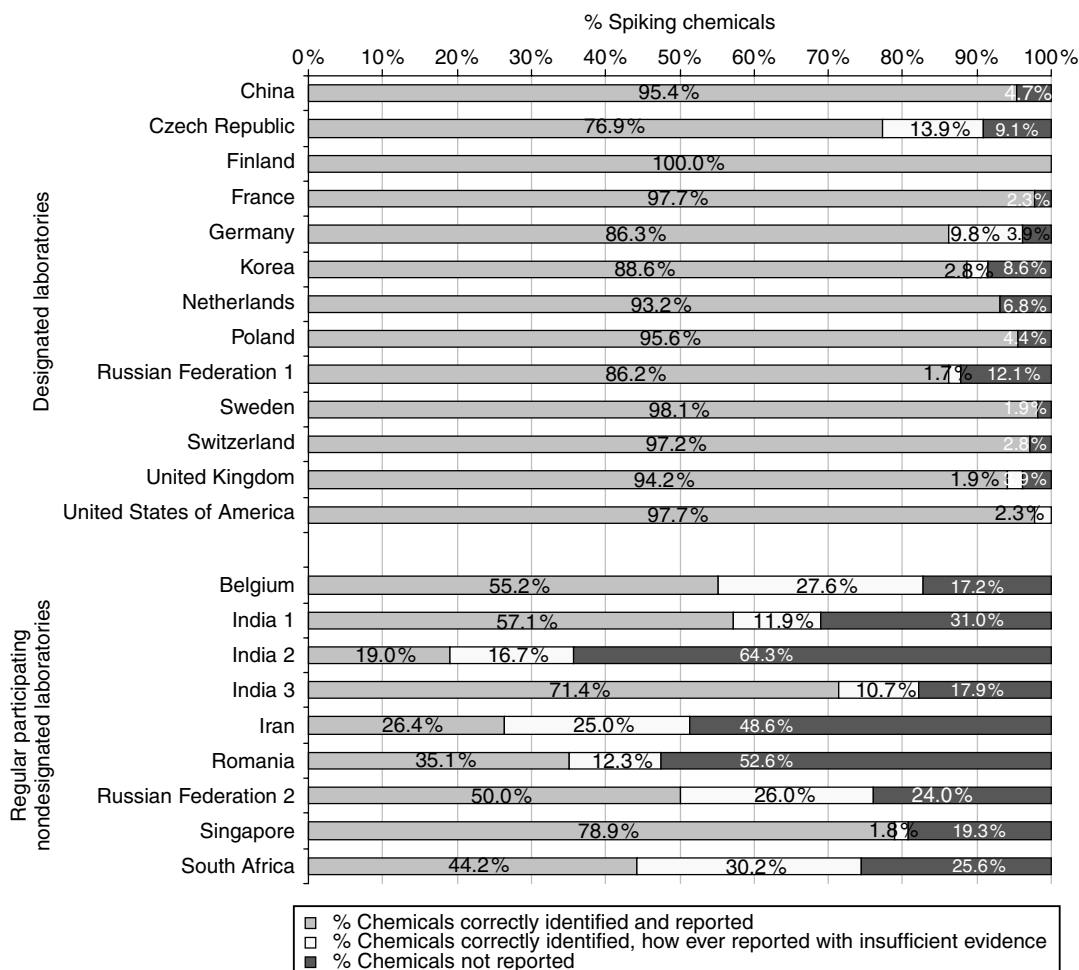


Figure 15. Overall performance of participants in proficiency tests 1 to 10, measured in the percentage of spiking chemicals

Three designated laboratories and most of the regularly participating nondesignated laboratories have reported false positive chemical(s). In addition, two regularly participating nondesignated laboratories have reported an irrelevant chemical. The laboratories and their respective performances in the area mentioned are shown in Figure 16.

Most of the designated laboratories have assisted the Technical Secretariat in conducting a proficiency test, either in preparing the test samples or in evaluating the test results. The type and frequency of assistance is shown in Figure 17. The regularly participating nondesignated laboratories have not assisted the Technical Secretariat

in any of these tasks up to the tenth proficiency test.

5.2 Scheduled Chemicals creating Difficulties

A more detailed study of the participants' performances revealed four groups of Scheduled chemicals that created difficulties in the identification for (some) participants:

1. Lewisite 1 (*syn.* 2-chlorovinylchloroarsine, CAS registry No. [541-25-3], Schedule 1.A.5);
2. Early and late eluting chemicals:

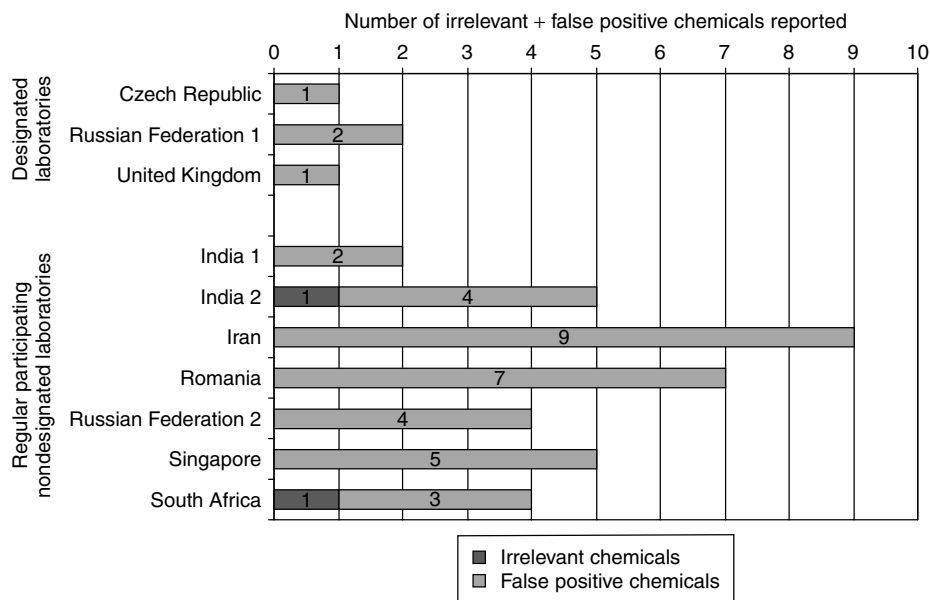


Figure 16. Overview of irrelevant and false positive chemicals, reported by participants in proficiency tests 1–10

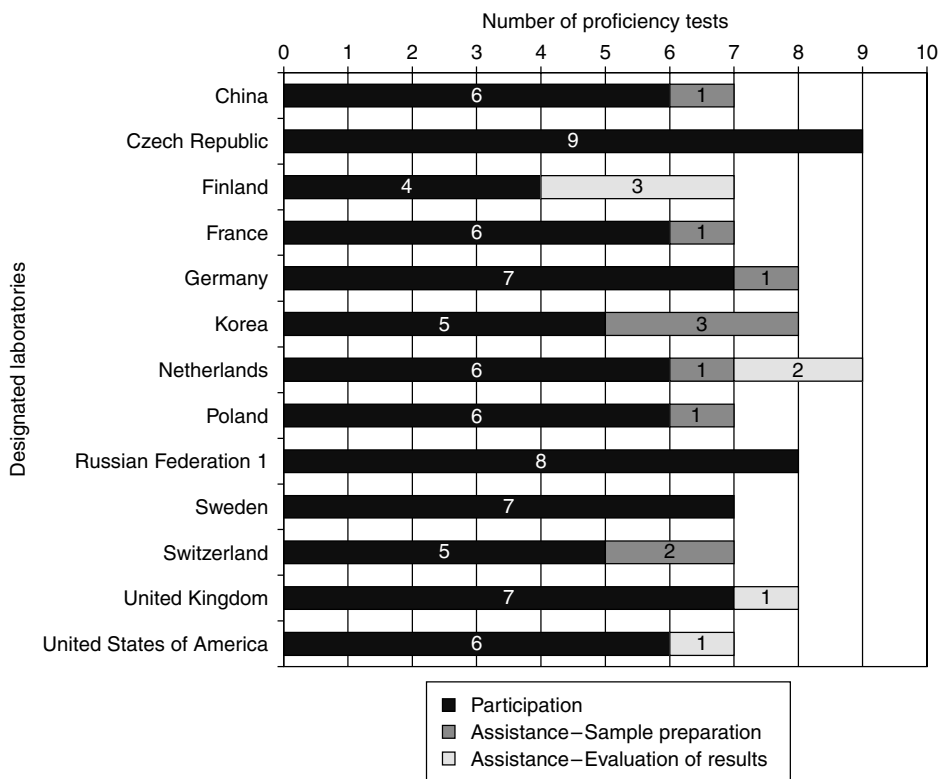


Figure 17. Frequency and type of participation of the designated laboratories in proficiency tests 1 to 10

- Pinacolyl alcohol, Retention Index 734.4 ± 2.1 (Retention Index pinacolyl alcohol is based on 6 measurements performed by the OPCW Laboratory), CAS registry No. [464-07-3], Schedule 2.B.14;
 - Chloropicrin, Retention Index 780.3 ± 1.8 (Retention Index chloropicrin is based on 6 measurements performed by the OPCW Laboratory), CAS registry No. [76-06-2], Schedule 3.A.04;
 - BZ (*syn.* 3-Quinuclidinyl benzilate), Retention Index 2658 (Retention Index BZ is taken from library: PDF-OCAD, version 4, October 2001), CAS registry No. [6581-06-2], Schedule 2.A.03
3. Chemicals not contained in a library at the time of the proficiency test (Libraries investigated are:
- The OPCW Central Analytical Database (OCAD), available for Member States to the CWC at OPCW, Johan de Wittlaan 32, 2517 JR The Hague, The Netherlands, <http://www.opcw.org>
 - The Wiley Registry of Mass Spectral Data, commercially available at John Wiley & Sons, Inc., <http://www.wiley.com/cda/product/0,,0471515930|desc|3047,00.html>
 - The NIST98-NIST/EPA/NIH Mass Spectral Library, commercially available at the National Institute of Standards and Technology, <http://www.sisweb.com/software/ms/nist98.htm>); and
4. Methylphosphonic acid (CAS registry No. [993-13-5], Schedule 2.B.4).

5.2.1 Lewisite 1

Lewisite 1 was used for spiking in the sixth and in the ninth proficiency test. The participants that failed to identify lewisite 1 did not describe an effective 'lewisite 1' sample preparation procedure⁽¹²⁾ in their analysis report, which could indicate that no such procedure had been employed. The participants' performances in the identification of lewisite 1 are summarized in Table 3.

Lewisite 1 per se is never found in the environment. Figure 18 shows that this compound hydrolyzes rapidly on contact with moisture to 2-chlorovinyl arsonous acid, which in turn slowly dehydrates to lewisite oxide (*syn.* 2-chlorovinyl arsonous oxide)⁽¹⁶⁾. Both 2-chlorovinyl arsonous acid and lewisite oxide are nonvolatile. The most frequently used method for the identification of CWC-related chemicals is based on gas chromatography (GC) in combination with mass spectrometry (GC/MS). Indirect GC/MS analysis of lewisite 1 requires sample preparation, which involves conversion of lewisite oxide to 2-chlorovinyl arsonous acid in an acidic environment, followed by derivatization⁽¹²⁾. The obtained species is both volatile and thermally stable, and thus amenable to GC analysis. Often, a mercaptan reagent is used as a derivatization agent. The reaction with 3,4-dimercaptotoluene is shown in Figure 19.

5.2.2 Early and Late Eluting Chemicals

Some participants had difficulty in the identification of the early eluting chemicals pinacolyl alcohol

Table 3. Participants' performances in the identification of lewisite 1

			Designated laboratories												Regularly participating nondesignated laboratories									
Chemical	Proficiency Test No.	Matrix	China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America	Belgium	India 1	India 2	India 3	Iran	Romania	Russian Federation 2	Singapore	South Africa
Lewisite 1	6	Organic	N	N	F	F	F	N	F	np	F	N	F	N	np	N	N	N	N	N	N	np	N	np
Lewisite 1	9	Decon	np	np	np	np	np	F	F	np	np	np	np	np	F	F	F	N	F	N	N	N	F	np

np Not participated in test

F Chemical correctly identified and reported

N Chemical not reported

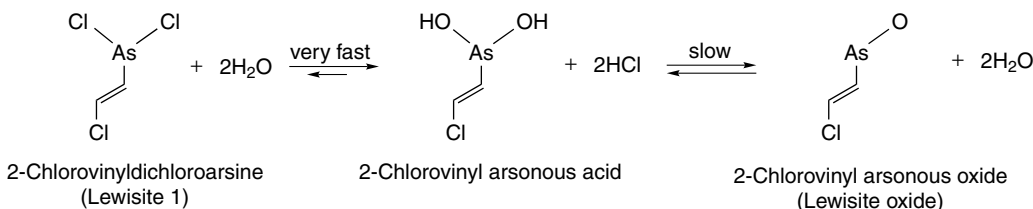


Figure 18. Conversion pathway for Lewisite 1 to related species ⁽¹⁶⁾

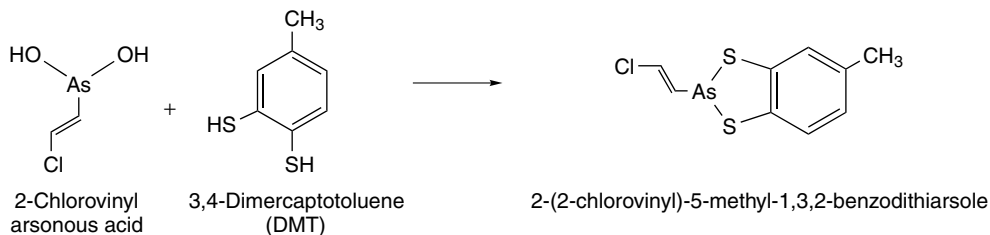


Figure 19. Formation of cyclic disulfide derivative of 2-chlorovinyl arsonous acid using 3,4-dimercaptotoluene ⁽¹⁶⁾

and chloropicrin, and/or the late eluting chemical 3-quinuclidinyl benzilate (BZ), because they either did not apply a proper sample preparation procedure or because they did not perform the chromatographic analysis under optimal conditions. The participants' performances in the identification of early and late eluting chemicals are summarized in Table 4.

Pinacolyl alcohol and chloropicrin are volatile, very stable in an aqueous environment, and easily extractable with an organic solvent. Exercise caution when evaporating the sample; the early eluting chemicals easily evaporate in this process.

BZ is nonvolatile, directly extractable with an organic solvent from an aqueous environment, and extractable with an aqueous solution from soil. Care should be taken in the attempt to extract BZ with an *organic solvent* from soil because efficiency of this extraction depends on the extraction conditions (e.g. temperature, pressure), the type of soil (e.g. sand, clay), and type of solvent (e.g. dichloromethane).

During chromatographic analysis, the early eluting chemicals are missed when, for example, the solvent delay time is set too long, or are easily overlooked

Table 4. Participants' performances in the identification of early and late eluting chemicals

Chemical	Proficiency Test No.	Matrix	Designated laboratories												Regularly participating nondesignated laboratories									
			China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America	Belgium	India 1	India 2	India 3	Iran	Romania	Russian Federation 2	Singapore	South Africa
Pinacolyl alcohol	1	Water	F	N	np	F	F	F	N	N	F	F	np	F	F	F	np	np	np	N	F	F	np	F
	9	Decon	np	np	np	np	np	F	F	np	np	np	np	np	F	F	np	N	N	N	F	F	N	np
Chloropicrin	9	Organic	np	np	np	np	np	N	N	np	np	np	np	np	F	N	N	N	N	N	N	N	N	np
BZ	4	Soil	np	F	np	np	F	np	N	F	F	np	np	F	F	N	np	N	N	N	F	F	F	F

np Not participated in test

F Chemical correctly identified and reported

N Chemical not reported

Table 5. Participants' performances in the identification of chemicals without reference data

	Designated laboratories												Regularly participating nondesignated laboratories									
	China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America	Belgium	India 1	India 2	India 3	Iran	Romania	Russian Federation 2	Singapore	South Africa
No chemical ^a tested on	16	25	13	13	19	12	18	22	20	22	14	23	18	25	13	14	19	27	21	19	22	14
No chemicals ^a FOUND	15	22	13	12	17	11	18	21	14	22	13	22	18	20	7	3	18	8	4	12	14	8
Overall Performance ^b (%)	94	88	100	92	89	92	100	95	70	100	93	96	100	80	54	21	95	30	19	63	64	57

^aChemicals for which no GC/EIMS library spectra was available in the OPCW Central Analytical Database (OCAD), the Wiley Registry of Mass Spectral Data and the NIST98-NIST/EPA/NIH

^aChemicals for which no GC/EIMS library spectra was available in the OPCW Central Analytical Database (OCAD), the Wiley Registry of Mass Spectral Data, and the NIST98-NIST/EPA/NIH Mass Spectral Library, at the time of the proficiency test

^bOverall performance in terms of the percentage of chemicals^a correctly identified and reported either with or without supportive evidence.

in case the system shows solvent tailing. The latter phenomenon, in particular, applies to chloropicrin, as chloropicrin shows identical electron impact mass spectrum to certain chlorinated solvents. An example is the electron impact mass spectrum of carbon tetrachloride, an impurity of solvent dichloromethane. In fact, this similarity in mass spectra can easily result in a false positive identification. The late eluting chemical, BZ, is missed when the total run time is set too short.

5.2.3 Chemicals without Reference Data

In the absence of library data, a laboratory must use its knowledge and experience in structure elucidation of CWC-related chemicals to find and identify chemicals of interest, and subsequently use its synthesis capabilities for confirmation. Structure elucidation is complicated by the presence of a background and by the low ppm level in which the spiking chemicals are present.

For 27 out of the 72 spiking chemicals, no library data was available at the time of the proficiency test in the OCAD, the Wiley Registry of Mass Spectral Data, and the NIST98-NIST/EPA/NIH Mass Spectral Library. Most designated laboratories seem to have little difficulty in the identification of these chemicals; the designated laboratories correctly identified 88–100 % of the chemicals, with one exception of 70 %. The opposite is true for the regularly participating nondesignated laboratories, with one exception; one regularly participating nondesignated laboratory correctly identified 95 % of these chemicals, which, in fact, is more than half of that achieved by the designated laboratories. The

participants' performances in the identification of chemicals without reference data are summarized in Table 5.

5.2.4 Methylphosphonic Acid

Methylphosphonic acid was used for spiking in the fifth and in the tenth proficiency test. Many participants, in particular, failed to identify methylphosphonic acid in the tenth test for reasons that are difficult to trace from their analysis reports. It can only be speculated that the high concentration of salt ($\approx 1\%$ NaCl) in the sample has been the cause; most of these participants did not describe an effective sample preparation procedure (e.g. cation exchange) for the removal of salts, and employed GC/MS as an indirect analysis technique. Indirect GC/MS analysis of methylphosphonic acid requires derivatization; salts are known to influence the derivatization reaction negatively. The participants' performances in the identification of methylphosphonic acid are summarized in Table 6.

5.3 Summary of Performance of Designated Laboratories

As of December 2002, the Technical Secretariat has designated 13 laboratories from three geographical groups: eight laboratories from the Western European and Others Group, three laboratories from the Eastern Europe Group, and two laboratories from the Asian Group. Three designated laboratories are temporarily suspended, because they performed unsuccessfully in a recent proficiency test. The designated laboratories and their

Table 6. Participants' performances in the identification of methylphosphonic acid

			Designated laboratories													Regularly participating nondesignated laboratories								
Chemical	Proficiency Test No.	Matrix	China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America	Belgium	India 1	India 2	India 3	Iran	Romania	Russian Federation 2	Singapore	South Africa
Methyl phosphonic acid	5	Water	F	F	np	F	F	np	np	np	F	F	np	F	np	np	F	N	F	F	F	F	F	F
	10	Decon	F	N	np	F	np	np	np	F	N	F	F	np	np	np	F	np	N	N	N	N	F	F

np

Not participated in test

F

Chemical correctly identified and reported

N

Chemical not reported

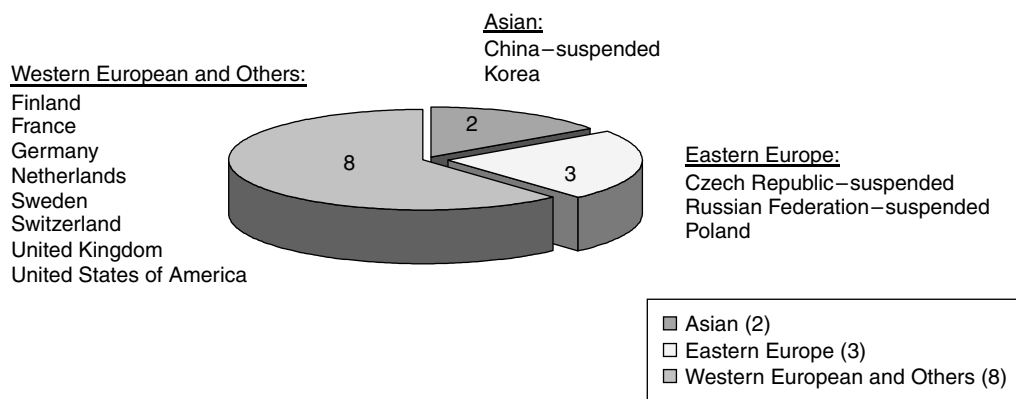


Figure 20. Designated laboratories and their geographical distribution, as of December 2002

Table 7. Summary of overall performance of designated laboratories

	China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America
Participation as regular participant	6	9	4	6	7	5	6	6	8	7	5	7	6
Assistance in sample preparation	1	0	0	1	1	3	1	1	0	0	2	0	0
Assistance in evaluating test results	0	0	3	0	0	0	2	0	0	0	0	1	1
Number of spiking chemicals tested on (max. 72)	43	66	29	44	51	35	44	45	58	52	36	52	43
Overall performance (%) ^a	95	91	100	98	96	91	93	96	88	98	97	96	98

^aOverall performance in terms of the percentage of chemicals found and reported *either* with *or* without supportive evidence.

geographical distributions are shown in Figure 20, listed in Annex 1, and their performance is summarized in Tables 7 and 8.

6 THE PROCESS OF EVALUATION, SCORING, AND DESIGNATION OF THE SCHEME

The final part of the OPCW proficiency-testing scheme involves a process of evaluation, scoring,

and designation. The evaluation process is an assessment of results, a method to judge the participants' reports in a single proficiency test. The outcome of the evaluation is used to score the participants; these scores are used to compare performances between laboratories in a single proficiency test. A sequence of scores, reflecting a laboratory's performance over a series of proficiency tests, is eventually used as a measure to determine whether a laboratory is qualified for obtaining and/or retaining the OPCW designation.

Table 8. Summary of strengths and weaknesses of designated laboratories in the identification of Scheduled chemicals

Schedule	Type of chemical	China	Czech Republic	Finland	France	Germany	Korea	Netherlands	Poland	Russian Federation 1	Sweden	Switzerland	United Kingdom	United States of America
1.A.1	Sarin/Soman-family	√	√	√	?	√	√	?	√	√	√	√	√	√
1.A.2	Tabun-family	√	√	?	√	√	√	√	√	√	√	?	√	√
1.A.3	VX-family	√	x	√	√	√	√	√	√	x	√	√	√	√
1.A.4	S-Mustards	√	√	√	√	√	√	√	√	√	√	√	√	√
1.A.5	Lewisites	x	x	√	√	√	x	√	?	√	x	√	x	√
1.A.6	N-Mustards	Not used as spiking chemical in proficiency test 1 to 10												
1.B	Precursors of schedule 1	Not used as spiking chemical in proficiency test 1 to 10												
2.A.1	Amiton	√	√	√	?	√	√	?	√	√	√	√	√	√
2.A.2	PFIB	Not used as spiking chemical in proficiency test 1 to 10												
2.A.3	BZ (late eluting chemical)	?	√	?	?	√	?	x	√	√	?	?	√	√
2.B.4	Alkylphosphonic acids: 1. Methylphosphonic acid	√	x	?	√	√	?	?	√	x	√	√	√	?
	2. library spectra available ^b	√	√	√	√	√	√	√	√	√	√	√	√	√
	3. no library spectra available ^{a,b}	√	√	√	√	√	√	√	x	√	√	√	√	√
	<i>O</i> -Alkyl alkylphosphonic acids	√	√	√	√	√	√	√	√	√	√	√	√	√
	<i>O</i> -Alkyl alkylphosphonothiolic acids ^{a,b}	?	√	?	?	√	?	√	√	x	?	?	√	√
	<i>O,O</i> -Dialkylalkylphosphonates: 1. library spectra available	√	√	√	√	√	√	√	√	√	√	√	√	√
	2. no library spectra available ^a	√	√	√	√	x	√	√	√	x	√	√	x	√
	<i>O,S</i> -Dialkylalkylphosphonothiolates	?	√	?	?	?	?	√	√	?	√	?	√	?
2.B.5	<i>N,N</i> -Dialkyl phosphoramidic dihalides	√	√	?	√	√	√	√	√	√	√	?	√	√
2.B.6	Dialkyl <i>N,N</i> -dialkylphosphoramidates	√	√	?	√	√	√	√	√	√	√	?	√	√
2.B.8	Benzylid acid (degradation product of BZ)	Not used as spiking chemical in proficiency test 1 to 10												
2.B.9	Quinuclidin-3-ol (degradation product of BZ)	Not used as spiking chemical in proficiency test 1 to 10												
2.B.10	<i>N,N</i> -Dialkyl aminoethane-2-chlorides	Not used as spiking chemical in proficiency test 1 to 10												
2.B.11	<i>N,N</i> -Dialkyl aminoethane-2-ols	?	√	√	√	√	√	√	√	√	?	√	√	√
2.B.12	<i>N,N</i> -Dialkyl aminoethane-2-thiols	Not used as spiking chemical in proficiency test 1 to 10												
2.B.13	Thiodiglycol	?	√	?	?	√	?	√	√	√	?	?	√	√
2.B.14	Pinacolyl alcohol (early eluting chemical)	√	x	?	√	√	√	x	x	√	√	?	√	√

(continued overleaf)

Table 8. (continued)

3.A.4	Chloropicrin (early eluting chemical)	?	?	?	?	?	x	x	?	?	?	?	?	?	√
3.B.8	Trimethyl phosphite	Not used as spiking chemical in proficiency test 1 to 10													
3.B.9	Triethyl phosphite	Not used as spiking chemical in proficiency test 1 to 10													
3.B.10	Dimethyl phosphite	√	√	?	√	?	?	?	√	√	√	√	?	?	?
3.B.11	Diethyl phosphite	Not used as spiking chemical in proficiency test 1 to 10													
3.B.15	Ethylthiolamine	√	√	√	√	√	√	√	?	√	√	√	√	?	?
3.B.16	Methylthiolamine	√	√	√	√	√	√	√	?	√	√	√	√	?	?
3.B.17	Triethanolamine	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Performance Designated laboratory:

√	Good :	Laboratory correctly identified all chemicals in this category it was tested on
?	Unknown :	Laboratory has not been tested on this category of chemicals
x	Weak :	Laboratory failed to identify at least one chemical in this category it was tested on

- ^a = Chemicals for which no GC/EIMS library spectra was available in the OPCW Central Analytical Database (OCAD), the Wiley Registry of Mass Spectral Data, and the NIST98-NIST/EPA/NIH Mass Spectral Library, at the time of the proficiency test
- ^b = Library spectrum for Trimethylbutyl- (TMS) and/or *tert*-Butyldimethylsilyl-(TBDMS) and/or Methyl-derivative of the original phosphonic acid

6.1 Process Description and Problems

The process of evaluation, scoring, and designation begins when the 15-calendar-days analysis and reporting period for all participants has expired. This timeline is the first criteria participants have to meet; an analysis report submitted within the 15 days will be considered for evaluation, whereas exceeding this timeline immediately results in failure of the test.

Prior to the evaluation, all test chemicals reported by the participants are categorized into (1) spiking chemicals, (2) chemicals, such as impurities, which are not used for scoring, (3) irrelevant chemicals, or (4) false positive chemicals. Spiking chemicals and 'non-scorable' chemicals will undergo full evaluation, whereas the reporting of irrelevant and/or false positive chemicals immediately results in failure of the test.

The evaluation is performed in accordance with the evaluation criteria specified in the quality system document 'QDOC/LAB/WI/PRO003⁽⁸⁾'. Reported spiking chemicals that meet the criteria are accredited with '+1' points, whereas spiking chemicals that do not (fully) meet the criteria, *for any reason*, are penalized with '-1' points. The latter penalty is also used for false negative results.

The sum of all points scored eventually represents a laboratory's performance in a single proficiency test. The maximum achievable points, however, depends on the total number of spiking chemicals used. As this number varies from test to test, the points scored cannot be used to compare performances between laboratories participating in different proficiency tests. This comparison is achieved with the final letter scoring system, and is shown in Table 9.

A sequence of the three most recent scores is used as measure to monitor a participant's trend in performance, and to decide upon a participant's status of designation. A sequence of either *three A's*, or *two A's and one B*, regardless of the order of scores, can lead to *designation*, provided that:

1. the laboratory has a quality system in place for the analysis of CWC-related chemicals;
2. the laboratory has obtained accreditation for the analysis of CWC-related chemicals by its national accreditation body accordingly; and
3. the scores reflect, at least, annual participation.

Once designated, a laboratory must *retain* this designation. The criteria for retaining designation are essentially the same as those that are applicable to

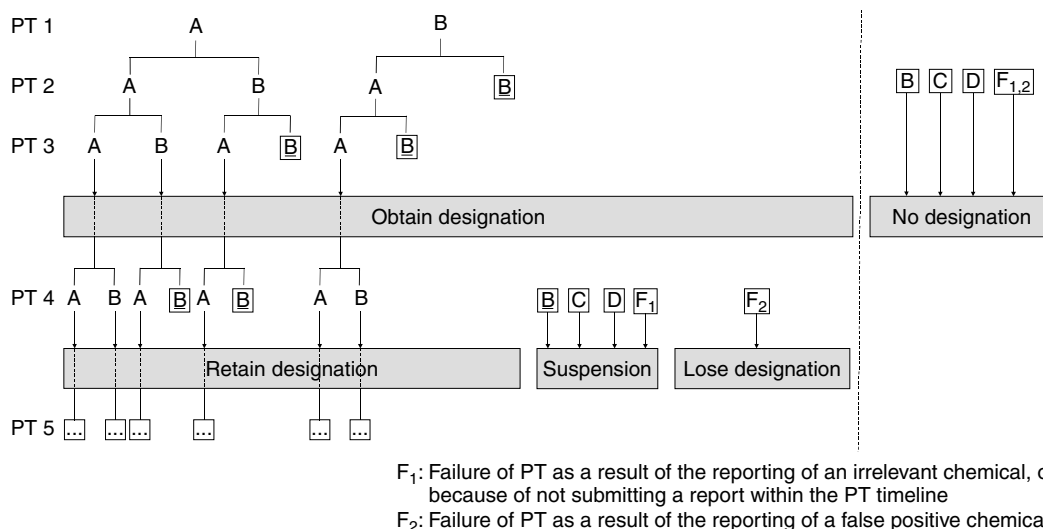
Table 9. Scoring system of the OPCW proficiency-testing scheme

Score	Explanation
A	Maximum achievable score. All spiking chemicals in the test have been identified and the identifications are supported with sufficient (analytical) data
B	Maximum score – 2. One spiking chemical in the test has either not been identified or has been identified but the identification was not supported with sufficient supportive (analytical) data.
C	Score between 0 and (Maximum score – 2). Between two and less than half of the spiking chemicals in the test have either not been identified or have been identified but the identifications were not supported with sufficient supportive (analytical) data.
D	Minimum score (<0). At least half of the spiking chemicals in the test have not been identified or have been identified but the identifications were not supported with sufficient supportive (analytical) data.
F	Failure. An irrelevant chemical or a false positive chemical has been reported, or no report has been submitted within the test timeline.

laboratories seeking designation, listed above. Any sequence other than three A's or two A's and one B, will either lead to *temporary suspension*, or will lead to immediate *loss of designation*:

- Temporary suspension in case of a second B, or a C, or a D, or an F score as a result of other than a false positive identification.
- Loss of designation in case of an F score as a result of a false positive identification.

In terms of analysis of authentic samples, there is no difference between temporary suspension and loss of designation; a laboratory will not be considered for this task until it has regained its designation. However, there *is* a difference when other tasks are concerned; a temporarily suspended laboratory may still be considered for assistance in tasks, which are defined in C-I/DEC.67 ('Decision, Scope of Activities of Designated Laboratories, and the Role and Status of Other Laboratories'), dated May 22, 1997.

**Figure 21.** Score possibilities and their consequences for designation

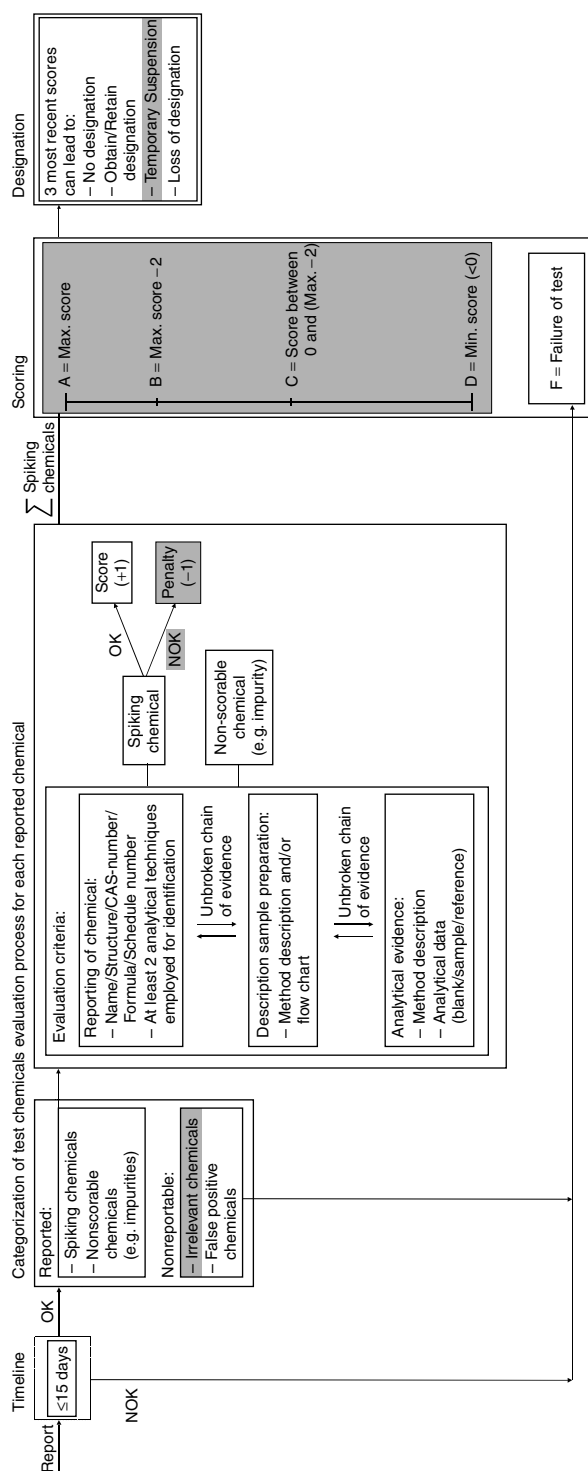


Figure 22. Process of evaluation, scoring, and designation of the OPCW proficiency-testing scheme

Some score possibilities and their consequences for designation, are shown in Figure 21.

The process of evaluation, scoring, and designation of the OPCW proficiency-testing scheme is shown in Figure 22. The process is known to have three weaknesses, the areas of weakness are highlighted in Figure 22 and summarized in the following:

1. Categorization of test chemicals – relevancy of chemicals

The guidelines for the relevancy of chemicals have been interpreted differently in the past (see Section 3.2), often leading to confusion among the participants. Clarification of the relevancy of chemicals is needed to avoid more confusion in future.

2. Scoring system

The scoring system does not make a distinction between different types of errors. The same penalty is always applied because any deviation from the reporting requirements is considered to lead to ambiguity, as shown in Figure 23.

The current penalty system is strict, clear, and straightforward; however, it does not allow for minor errors. In fact, minor errors are treated equally to a false negative result, as demonstrated with the following example: Laboratory 1 failed to identify one of the spiking chemicals in a test. Laboratory 2 found all spiking chemicals; however, it made a minor reporting error in the identification of one of them; the evidence provided was adequate to indicate the presence of the particular chemical, however, did not fulfill all the reporting requirements.

With the current scoring system, both laboratories receive the same penalty; the chemical is penalized with –1 points. A solution to this would be to create an additional penalty level, a milder penalty for minor errors, which do not lead to ambiguity.

The range for scoring varies from A (i.e. maximum score) to F (i.e. failure of the test). This ‘A, B, C... system’ allows for comparison of performance in a number of different proficiency tests, in a rather simple and unambiguous way. However, in combination with the previously discussed strict penalty system, the simplicity of this letter system is at the expense of its transparency; letter scores below the maximum score ‘A’ do not reflect the type of error(s) made.

3. Designation mechanism – temporary suspension Decision EC-XX/DEC.3 (EC-XX/DEC.3 ‘Decision, Guidelines on the Designation of Laboratories for the Analysis of Authentic Samples’, dated 28 June 2000) on the designation of laboratories for the analysis of authentic samples stipulates that temporarily suspended laboratories can lose designation when they fail to regain full designation status. However, no restriction, in terms of a timeline or a maximum number of proficiency tests after which full designation status must be regained, has been specified. The lack of such a restriction prevents this criterion from being implemented.

To improve the process of evaluation, scoring, and designation of the OPCW proficiency-testing

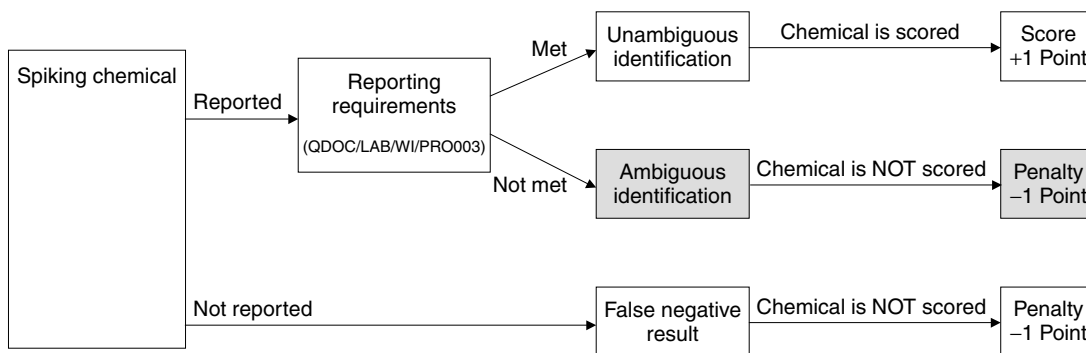


Figure 23. Simplified overview of evaluation and scoring process of the OPCW proficiency-testing scheme

scheme, it is recommendable to address the weaknesses identified. A discussion should be initiated to clarify the relevancy of chemicals, to implement an additional penalty level for minor errors, and to implement a time restriction in which temporarily suspended laboratories must regain full designation status. The design of the current scoring system does not allow for an additional penalty level unless the letter scores are redefined. An alternative could be to design an entire new scoring system with a corresponding designation mechanism instead.

7 DISCUSSION AND RECOMMENDATIONS

The Technical Secretariat of the Organization for the Prohibition of Chemical Weapons (OPCW) provides a proficiency-testing scheme for the analysis of samples in the context of the CWC. The design of the scheme should simulate analysis of authentic samples that are taken during inspections. The purpose of the scheme is to select, certify, and train highly competent laboratories for the analysis of CWC-related chemicals in various matrices. The Technical Secretariat designates laboratories, which perform successfully in the scheme, to support it in such analysis should they become necessary during the course of its verification activities.

7.1 Analysis and Reporting

Participation in the OPCW proficiency-testing scheme is not an easy task. The analysis of samples of different matrices is aimed at qualitatively identifying CWC-related chemicals of which there are millions of theoretical possibilities. The test combines sample preparation and analysis and detailed reporting of results. The analysis part, naturally, requires the availability of laboratory resources. The reporting is supported by the availability of reporting templates. In order to complete the test within the short timeline of 15 calendar days successfully, the application of an analysis and reporting strategy was found to be essential. A model for an analysis and reporting strategy has been presented. This model emphasizes the importance of a well-managed and structured

team approach among laboratory staff as well as commitment and support from other levels within a laboratory's organization, including management.

7.2 Test Suitability

The aim of the scheme, which is to simulate off-site sample analysis, is partially met. One important element, the set of samples, still reflects the old sampling philosophy, in which it was assumed that samples and matrix blanks were both taken from the inspection site. Anticipated problems with collecting true blanks on-site, and unknown effects of such blank matrices on spiking chemicals when preparing control samples changed the sampling philosophy. This was done, however, without updating the process of the proficiency-testing scheme. In order to address this difference between real off-site analysis and proficiency testing, it is recommended to change the design of the sample composition in the proficiency-testing scheme, to reflect the new philosophy.

Another element is the use of a 'cocktail' of different Scheduled chemicals in a single proficiency test. Applying a 'cocktail' of chemicals may not be very realistic, however, it is considered unavoidable in order to train participants in the analysis of as many different Scheduled chemicals as possible in a small number of tests. The chemicals, used for spiking the samples, have been reviewed, which revealed that certain Scheduled chemicals have not been covered in the first 10 proficiency tests. Therefore, it would be recommendable to include such chemicals in future scenarios. The same recommendation applies to several identified categories of chemicals, which *have* been applied in previous proficiency tests, but created difficulties (repeatedly) for some of the designated laboratories, as well as for most of the nondesignated laboratories that regularly participate in the scheme.

There were differences identified in the degree of difficulty between the first 10 proficiency tests. These differences are a result of the changing sample composition, that is, different matrices, spiking chemicals, and background materials. It is mainly a laboratory's experience in the analysis of CWC-related chemicals that seems to allow the participants to cope with these varying challenges. The Technical Secretariat tries to maintain a similar degree

of difficulty between proficiency tests, by controlling the sample composition for each individual test, in order to create a fair and consistent proficiency-testing scheme. However, not all problems that participants may face when analyzing the samples can be anticipated in advance of a test.

There is one element within the proficiency test process that causes difficulties for many of the participants. The proficiency-testing scheme trains participants to *not* report chemicals that are considered irrelevant within the scenario of the test and which, in real off-site analysis, could reveal confidential information that is considered not relevant in the context of the CWC. A review on how the test scenarios were applied revealed that participants are not really in a position to judge the relevancy of chemicals. It rather should be the *Technical Secretariat* making a final decision on the relevancy of chemicals instead, and, for that reason, accept the reporting of *any* chemical given that it is a degradation product of a Scheduled chemical or directly related to a Scheduled chemical.

The current test criteria on reporting irrelevant chemicals in fact would allow such reporting. The problem, however, is that this definition has been interpreted differently in the past, leading to confusion sometimes. The current penalty is severe; the reporting of irrelevant chemicals leads to immediate failure of the test. This penalty may, in fact, be too severe considering that Paragraph C.17 of the Convention's Confidentiality Annex allows the Technical Secretariat to process information in the inspection report into less sensitive forms before transmitting it outside. However, 'filtering' of information is a matter that should be done with permission from the inspected State Party only. It is clear that the entire matter on the relevancy of chemicals must be reviewed to improve transparency of the scheme in future.

7.3 Evaluation, Scoring, and Designation Process

Besides the categorization of irrelevant chemicals, other areas within the process of evaluation, scoring, and designation were identified to have weaknesses. The *evaluation process* is very strict; the criteria are either *met* or are *not met*; there is no option in between. As a consequence, the same penalty is

applied for various types of errors, which could be perceived as unfair by participants, in particular, if a compound has been identified with minor errors, but is scored like it had not been reported at all. Identifications with 'minor' errors could still be of value to the Technical Secretariat, provided that the errors do not lead to technical ambiguity about the correctness of the identification.

The *criteria for designation* of laboratories in EC-XX/DEC.3 are incomplete; once designated always designated, there is no timeline defined during which full designation status must be regained if a laboratory that is designated has been temporarily suspended.

7.4 Test Participants

As a result of the first ten official OPCW proficiency tests, the Technical Secretariat has designated 13 laboratories out of the approximately 22 regular participants worldwide. The performance of these 22 laboratories has been studied in detail, which revealed areas of strengths and weaknesses for each of them. The latter information may become useful for the Technical Secretariat when selecting designated laboratories for the analysis of authentic samples and when identifying particular training requirements.

7.5 Closing Remarks

It is an objective of the OPCW, as decided by the Conference of the States Parties, to have designated laboratories from a wide geographical distribution. At present, the African group is participating with one laboratory and the Latin American and Caribbean group has no regular participant. It is recommended to arouse (new) laboratories' interest in participation in order to obtain designated laboratories from all regional groups.

ACKNOWLEDGMENT

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ABBREVIATIONS AND ACRONYMS

1D-NMR	One-dimensional Nuclear Magnetic Resonance
AED	Atomic Emission Detector
APCIMS	Atmospheric Pressure Chemical Ionization Mass Spectrometry
CAS	Chemical Abstracts Service
CA	Chemical Abstract
CE/FPD	Capillary Electrophoresis with Flame Photometric Detection
CWC	Chemical Weapons Convention
EIC	Extracted Ion Chromatogram
EIMS	Electron Impact Mass Spectrometry
ESI	Electrospray Ionization
FIA	Flow Injection Analysis
FPD	Flame Photometric Detector
GC/FTIR	Gas Chromatography/Fourier Transform Infrared Spectroscopy
GC/MS	Gas Chromatography/Mass Spectrometry
GC	Gas chromatography
IUPAC	International Union for Pure and Applied Chemistry
LC/MS(MS)	Liquid Chromatography/Tandem Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NPD	Nitrogen–Phosphorus Detector
OCAD	OPCW Central Analytical Database
OPCW	Organization for the Prohibition of Chemical Weapons
TIC	Total Ion Chromatogram
VERIFIN	Finnish Institute for Verification of the Chemical Weapons Convention

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ANNEX 1. LIST OF DESIGNATED LABORATORIES NETWORK

Table 10. List of Designated Laboratories Network as of December 2002

States Party	Laboratory	Date of Designation	Designation Status
China	The Laboratory of Analytical Chemistry	17-Nov-1998	Suspended*
Czech Republic	Research Institute of Organic Syntheses, Centre of Ecology, Toxicology and Analytics, Analytical Department	29-Jun-1999	Suspended
Finland	Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN)	17-Nov-1998	Designated
France	Centre d'Etudes du Bouchet (CEB)	29-Jun-1999	Designated
Germany	Wehrwissenschaftliches Institut für Schutztechnologien – ABC-Schutz	29-Jun-1999	Designated
Netherlands	TNO Prins Maurits Laboratory	17-Nov-1998	Designated
Republic of Korea	Chemical Analysis Laboratory, CB Department, Agency for Defence Development	17-Nov-1998	Designated
Republic of Poland	Laboratory for Chemical Weapons Convention Verification, Military Institute of Chemistry and Radiometry	29-Jun-1999	Designated
Russian Federation	The Laboratory for the Chemical and Analytical Control of Military University for the Radioactive, Chemical and Biological Protection	4-Aug-2000	Suspended**
Sweden	Swedish Defence Research Agency (FOI)	17Nov. 1998	Designated
Switzerland	Spiez Laboratory	17-Nov-1998	Designated
United Kingdom	Defence Science and Technology Laboratory, Porton Down	29-Jun-1999	Designated
United States of America	Edgewood Chemical and Biological Forensic Analytical Center	17-Nov-1998	Designated

* These designated laboratories performed unsuccessfully in the sixth official OPCW proficiency test

** This designated laboratory performed unsuccessfully in the tenth official OPCW proficiency test

ANNEX 2. LIST OF COMMERCIALY AVAILABLE SCHEDULED COMPOUNDS, ALCOHOLS AND THIOALCOHOLS

Table 11. Commercially available Schedule 2 compounds

Chemical name	CAS number	Supplier*
Methylphosphonyl dichloride	676971	Sigma Aldrich
Ethylphosphonyl dichloride	1066508	Sigma Aldrich
Propylphosphonyl dichloride	4708047	Sigma Aldrich
Isopropylphosphonyl dichloride	1498460	Radian International LLC
Methylphosphonothioic dichloride	676982	Sigma Aldrich
<i>N,N</i> -Dimethylphosphoramidic dichloride	677430	Sigma Aldrich
Dimethyl methylphosphonate	756796	Sigma Aldrich
Dimethyl ethylphosphonate	6163753	Sigma Aldrich
Diethyl methylphosphonate	683089	Sigma Aldrich
Diisopropyl methylphosphonate	1445756	Radian International LLC
Diethyl ethylphosphonate	78386	Sigma Aldrich
Bis(2-chloroethyl) methylphosphonate	2799588	Sigma Aldrich
<i>O,O'</i> -Diethyl methylphosphonothioate	6996812	Sigma Aldrich
Methyl methylphosphonate	1066531	Radian International LLC
Ethyl methylphosphonate	1832537	Sigma Aldrich
Isopropyl methylphosphonate	1832548	Radian International LLC
<i>O</i> -Ethyl methylphosphonothioate	18005408	Sigma Aldrich
Pinacolyl methylphosphonate	616524	Sigma Aldrich
Cyclohexyl methylphosphonate	1932601	Radian International LLC
Methylphosphonic acid	993135	Sigma Aldrich
Ethylphosphonic acid	6779095	Sigma Aldrich
Propylphosphonic acid	4672382	Sigma Aldrich
Diethylamino ethanethiol *HCl	1942525	Sigma Aldrich
Dimethylamino ethanethiol *HCl	13242449	Sigma Aldrich
2-(Diethylamino)-ethanethiol	100389	Merck
2-(Diisopropylamino)ethanol	96800	Sigma Aldrich
2-(Dimethylamino)ethyl chloride*HCl	4584467	Sigma Aldrich
2-(Diethylamino)ethyl chloride*HCl	869249	Sigma Aldrich
2-(Diisopropylamino)ethyl chloride*HCl	4261681	Sigma Aldrich
Arsenic trichloride	7784341	Sigma Aldrich
Benzilic acid	76937	Sigma Aldrich
3-Quinuclidinol	1619347	Sigma Aldrich
Thiodiglycol	111488	Sigma Aldrich
Pinacolyl alcohol (3,3-Dimethylbutan-2-ol)	464073	Sigma Aldrich

*Sigma Aldrich: <http://www.sigma-aldrich.com>; Merck: <http://www.merck.com>;
Radian: <http://www.radian.com>

Table 12. Commercially available Schedule 3 compounds

Chemical name	CAS number	Supplier*
Chloropicrin	76062	Sigma Aldrich
Phosphorus oxychloride	10025873	Sigma Aldrich
Phosphorus trichloride	7719122	Sigma Aldrich
Trimethyl phosphite	121459	Sigma Aldrich
Triethyl phosphite	122521	Sigma Aldrich
Dimethyl phosphite	868859	Sigma Aldrich
Diethyl phosphite	762049	Sigma Aldrich
Thionyl chloride	7719097	Sigma Aldrich
Ethyl diethanolamine	139877	Sigma Aldrich
Methyl diethanolamine	105599	Sigma Aldrich
Triethanolamine	102716	Sigma Aldrich

*Sigma Aldrich: <http://www.sigma-aldrich.com>; Merck: <http://www.merck.com>; Radian: <http://www.radian.com>

Table 13. Commercially available degradation products of Schedule 1.A.4 compounds

Chemical name	CAS number	Supplier*
2-Hydroxyethyl disulfide	1892291	Sigma Aldrich
Thiodiglycolic acid	123933	Sigma Aldrich
1,4-Dithiane	505293	Sigma Aldrich
1,4-Thioxane	15980151	Sigma Aldrich
2-Chloroethyl methyl sulfide	542814	Sigma Aldrich
2-Chloroethyl ethyl sulfide	693072	Sigma Aldrich
Thiodiglycol sulfoxide	3085458	Radian International LLC

*Sigma Aldrich: <http://www.sigma-aldrich.com>; Merck: <http://www.merck.com>; Radian: <http://www.radian.com>

Table 14. Commercially available thioalcohols

Chemical name	CAS number	Supplier*
Ethanethiol	75082	Sigma Aldrich
Ethanethiol sodiumsalt	811518	Sigma Aldrich
1-Propanethiol	107039	Sigma Aldrich
2-Propanethiol	75332	Sigma Aldrich
1-Butanethiol	109795	Sigma Aldrich
2-Butanethiol	513531	Sigma Aldrich
1-Pentanethiol	110667	Sigma Aldrich
Cyclopentyl mercaptan	1679078	Sigma Aldrich
1-Hexanethiol	111319	Sigma Aldrich
Cyclohexyl mercaptan	1569693	Sigma Aldrich
1-Heptanethiol	1639094	Sigma Aldrich
1-Octanethiol	111886	Sigma Aldrich
1-Nonanethiol	1455216	Sigma Aldrich
1-Decanethiol	143102	Sigma Aldrich
1-Dodecanethiol	112550	Sigma Aldrich

*Sigma Aldrich: <http://www.sigma-aldrich.com>; Merck: <http://www.merck.com>; Radian: <http://www.radian.com>

Table 15. Commercially available alcohols

Chemical name	CAS number	Supplier*
Methanol	–	Sigma Aldrich
Ethanol	–	Sigma Aldrich
Propanol	–	Sigma Aldrich
2-Propanol	–	Sigma Aldrich
1-Butanol	71363	Sigma Aldrich
2-Butanol	78922	Sigma Aldrich
Cyclobutanol	2919235	Sigma Aldrich
1-Pentanol	71410	Sigma Aldrich
2-Pentanol	6032297	Sigma Aldrich
3-Pentanol	584021	Sigma Aldrich
2-Methyl-1-butanol	137326	Sigma Aldrich
2-Methyl-2-butanol (tert-Amyl alcohol)	75843	Sigma Aldrich
3-Methyl-2-butanol	598754	Sigma Aldrich
3-Methyl-1-butanol (iso-Amyl alcohol)	123513	Sigma Aldrich
2,2-Dimethyl-1-propanol (Neopentyl alcohol)	75843	Sigma Aldrich
Cyclopentanol	96413	Sigma Aldrich
Cyclobutanemethanol	4415821	Sigma Aldrich
1-Hexanol	111273	Sigma Aldrich
2-Hexanol	626937	Sigma Aldrich
3-Hexanol	623370	Sigma Aldrich
2-Methyl-1-pentanol	105306	Sigma Aldrich
2-Methyl-2-pentanol	590363	Sigma Aldrich
2-Methyl-3-pentanol	565673	Sigma Aldrich
3-Methyl-2-pentanol	565606	Sigma Aldrich
3-Methyl-3-pentanol	77747	Sigma Aldrich
3-Methyl-1-pentanol	589355	Sigma Aldrich
4-Methyl-2-pentanol	108112	Sigma Aldrich
4-Methyl-1-pentanol	626891	Sigma Aldrich
3,3-Dimethyl-1-butanol	624953	Sigma Aldrich
2,3-Dimethyl-2-butanol	594605	Sigma Aldrich
2-Ethyl-1-butanol	97950	Sigma Aldrich
Cyclohexanol	108930	Sigma Aldrich
1-Methylcyclopentanol	1462039	Sigma Aldrich
Cyclopentanemethanol	3637614	Sigma Aldrich
3-Methylcyclopentanol	18729481	Sigma Aldrich
1-Heptanol	111706	Sigma Aldrich
2-Heptanol	543497	Sigma Aldrich
3-Heptanol	589822	Sigma Aldrich
2-Methyl-2-hexanol	625230	Sigma Aldrich
2-Methyl-3-Hexanol	617298	Sigma Aldrich
5-Methyl-2-hexanol	627598	Sigma Aldrich
2,4-Dimethyl-3-pentanol	600362	Sigma Aldrich
2,2-Dimethyl-3-pentanol	3970625	Sigma Aldrich
2,3-Dimethyl-3-pentanol	595415	Sigma Aldrich
4,4-Dimethyl-2-pentanol	6144930	Sigma Aldrich
3-Ethyl-3-pentanol	597499	Sigma Aldrich
Cycloheptanol	502410	Sigma Aldrich
1-Methylcyclohexanol	590670	Sigma Aldrich
2-Methylcyclohexanol	583595	Sigma Aldrich
3-Methylcyclohexanol	591231	Sigma Aldrich
4-Methylcyclohexanol	589913	Sigma Aldrich
Cyclohexylmethanol	100492	Sigma Aldrich
1-Octanol	111875	Sigma Aldrich
2-Octanol	123966	Sigma Aldrich
3-Octanol	20296291	Sigma Aldrich

(continued overleaf)

Table 15. (continued)

Chemical name	CAS number	Supplier*
4-Methyl-3-heptanol	14979396	Sigma Aldrich
6-Methyl-2-heptanol	4730227	Sigma Aldrich
2-Ethyl-1-hexanol	104767	Sigma Aldrich
2,4,4-Trimethyl-1-pentanol	16325636	Sigma Aldrich
2-Propyl-1-pentanol	58175578	Sigma Aldrich
Cyclooctanol	696719	Sigma Aldrich
Cycloheptanemethanol	4448753	Sigma Aldrich
2,6-Dimethylcyclohexanol	5337724	Sigma Aldrich
2,3-Dimethylcyclohexanol	1502245	Sigma Aldrich
3,5-Dimethylcyclohexanol	5441521	Sigma Aldrich
2-Ethylcyclohexanol	3760201	Sigma Aldrich
4-Ethylcyclohexanol	4534741	Sigma Aldrich
1-Cyclohexylethanol	1193813	Sigma Aldrich
2-Cyclohexylethanol	4442799	Sigma Aldrich
3-Cyclopentyl-1-propanol	767055	Sigma Aldrich
1-Nonanol	143088	Sigma Aldrich
2-Nonanol	628999	Sigma Aldrich
2,6-Dimethyl-4-heptanol	108827	Sigma Aldrich
3,5,5-Trimethyl-1-hexanol	3452979	Sigma Aldrich
3-Ethyl-2,2-dimethyl-3-pentanol	66793962	Sigma Aldrich
3-Cyclohexylpropanol	1124636	Sigma Aldrich
1-Decanol	112301	Sigma Aldrich
2-Decanol	1120065	Sigma Aldrich
4-Decanol	2051312	Sigma Aldrich
3,7-Dimethyl-1-octanol	106218	Sigma Aldrich
3,7-Dimethyl-3-octanol	78693	Sigma Aldrich
4-Cyclohexyl-1-butanol	4441570	Sigma Aldrich
2-tert-Butylcyclohexanol	13491797	Sigma Aldrich
4-tert-Butylcyclohexanol	98522	Sigma Aldrich
3,3,5,5-Tetramethylcyclohexanol	2650400	Sigma Aldrich
1-Undecanol	112425	Sigma Aldrich
2-Undecanol	1653301	Sigma Aldrich
4-tert-Amylcyclohexanol	5349519	Sigma Aldrich
1-Dodecanol	112538	Sigma Aldrich
2-Methoxyethanol	109864	Sigma Aldrich
2-Ethoxyethanol	110805	Sigma Aldrich
1-Methoxy-2-propanol	107982	Sigma Aldrich
2-Propoxyethanol	2807309	Sigma Aldrich
3-Methoxy-1-butanol	2517433	Sigma Aldrich
3-Ethoxy-1-propanol	111353	Sigma Aldrich

*Sigma Aldrich: <http://www.sigma-aldrich.com>; Merck: <http://www.merck.com>; Radian: <http://www.radian.com>

CHAPTER 7

The OPCW Central Analytical Database

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1 INTRODUCTION

The OPCW Central Analytical Database (OCAD) is compiled and maintained by the OPCW Laboratory and contains analytical data of the chemicals that fall under the Chemical Weapons Convention (CWC) ⁽¹⁾. States Parties (SPs) of the CWC and the OPCW (Organization for Prohibition of the Chemical Weapons) Laboratory submit analytical data for inclusion to the OCAD. These analytical data are peer reviewed by experts. Before the analytical data are included into the OCAD, they undergo a technical and political approval process.

The main purpose of the OCAD is to support the verification activities of the Technical Secretariat (Secretariat) as provided for in the CWC. Copies of the OCAD are made available to all States Parties.

The analytical data in the OCAD is derived from four different techniques. These are nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), gas chromatography/mass spectrometry (GC/MS), and gas chromatography retention indices (GC(RI)). With a few exceptions, the OCAD contains only data of compounds that are listed in the schedules of the CWC and their derivatives of BSTFA and dimercaptotoluene.

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

Edited by Markku Mesilaakso.

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There are two main decisions of the Conference of States Parties (CSP), which regulate the work of the OCAD. These are the 'Proposed Mechanism for Updating the OPCW CAD' (EC-IV/DEC.2) dated September 5, 1997, and adopted by the second Conference of the States Parties (C-II/8 paragraph 11.2 (c)) ⁽²⁾, and the 'Authentication and certification procedure for the OPCW CAD and on-site databases' (C-VI/DEC.4, dated May 17, 2001) ⁽³⁾.

2 SCOPE OF THE OCAD

The scope is limited to the schedule list (schedule 1, 2, and 3) in the Annex on Chemicals. The nature of the chemicals is described in Article II of the CWC. Article II defines the chemicals covered by the CWC and gives the criteria that determine if a chemical within a given context can be included or excluded from the scope for the purposes of the CWC.

The definitions and criteria that determine whether a chemical falls within the scope of the CWC or not, leaves a large number of chemicals in the gray area. One example of a group of chemicals that are excluded from the OCAD on the basis of 'purposes not prohibited under this Convention', is given by the 'Riot Control Agents'. There are other examples, which need to be addressed in the future to determine how they can be regulated by the CWC as these have been seen to be very useful in the verification activities of the OPCW. Efforts are underway to find a way for the inclusion in the OCAD, of chemicals that are relevant to the CWC but are not covered by the schedule list (Scientific Advisory Board (SAB) and Validation Group (VG)).

3 HISTORICAL DEVELOPMENT

During the Preparatory Commission (PC) for the OPCW, a group of experts for the purposes of evaluating newly measured analytical data, was formed and was known as the Task Force of the Analytical Databases. These experts were nominated by their Governments and were all from the States Parties of the OPCW. The first meeting was organized by the Secretariat with the experts from those Member States that provided data, in March 1994.

The Specialist Task Force on Analytical Databases established the mechanism of peer review of data for its inclusion into the OCAD. They also established the evaluation criteria for each type of data to be included in the OCAD, which they used as the basis for evaluating and accepting data. The Task Force reviewed each datum (spectrum) for technical validity. During the Preparatory Commission time, The Task Force recommended to the Expert Group on Inspection Procedures to approve the data the Task Force had evaluated and accepted. The PC would formally approve the data, which was later adopted by the CSP (see Figure 1). The current approval process is illustrated alongside the PC process for comparison purposes.

In the early days, the analytical data contributed to the Secretariat for the purposes of developing the OCAD, was printed on paper, and this data was referred to as the hard copy version. The evaluation was based on the hard copy version. This data was evaluated, once accepted, the digital analytical data (electronic version) corresponding to the hard copies was then requested from the contributing Member States at a later stage, sometimes a year later. This applied only to the MS (Mass Spectra) and IR analytical data. The problem with this procedure

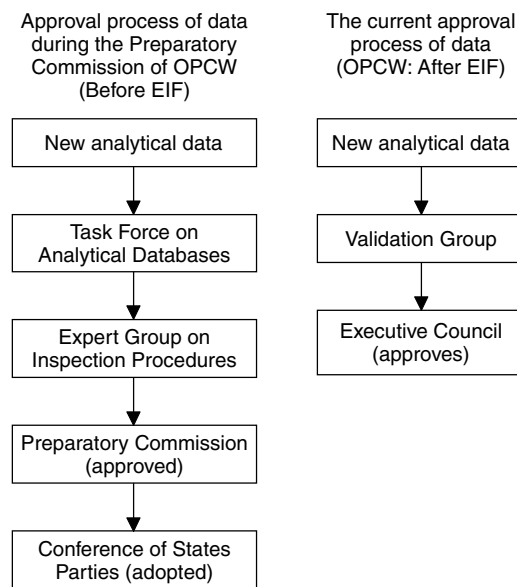


Figure 1. Comparison of the bodies approving analytical data for inclusion into OCAD before and after 'Entry into Force' (EIF) of the CWC

was that, by the time the electronic version was requested, the original electronic version may have been lost or corrupted and the only option then would be to reacquire similar data if conditions allow. The end result was that the electronic data provided at a later stage (long after the evaluation process) would be different from the validated hard copy version of the data. In the case of NMR, only hard copy data was and still is the form of the data in the OCAD. In cases where more than one datum of a particular compound were available, they would be incorporated into the OCAD.

After Entry into Force (EIF) of the CWC, the Director-General established a new group, named the VG, comprising the participants of the Task Force of the Analytical Databases and other experts from States Parties of the OPCW. The VG, after EIF, replaced the Task Force of the Analytical Databases.

The practise of requesting the electronic version from the Contributor after the analytical data, on hard copy, had already been validated, continued till 2000. In order to avoid the problem of inconsistent data between electronic and hard copy version, the VG decided in 2000, that starting from January 1, 2001 onward the validation process of MS data would be based on the electronic version. Also from the same time, both the hard copy and the electronic version were to be submitted for evaluation at the same time. In case of inconsistency in the case of MS data, the electronic version of each spectrum would be considered correct and not the hard copy version. This new approach has been implemented successfully for the MS analytical technique.

During the Preparatory Commission and the first couple of years after EIF, the corresponding electronic version obtained from the Contributor was sent to the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN (Finnish Institute for Verification of the Chemical Weapons Convention) actively participated in the building of the electronic version of the OCAD.) for incorporation into the electronic version of the OCAD. The original idea was to include all the different types of analytical data in a single relational database. The database developed by VERIFIN is a relational database (VERIFY) (for more information on the VERIFY database, contact VERIFIN (Finland)), which contains all the different

types of data, including NMR in electronic format. However, this database is based on the specifications and requirements of VERIFIN and not on the requirements of the Secretariat. Only one updateable version of the VERIFY database is kept at VERIFIN. All modifications are made to this database. The electronic version of OCAD used to be prepared from the VERIFY database. The first electronic MS analytical database for on-site purposes, was extracted from the electronic version of the OCAD prepared from VERIFY database. However, this approach of incorporating OCAD data at VERIFIN, inadvertently combined the OPCW validated data with additional information already existing in the VERIFY database, which was not part of the validated OPCW data. In VERIFY, a field 'OPCW code' is filled in when the (spectral) data has been approved by the VG. The main problems with this approach, were:

- (1) Data and information not validated was included in the OCAD;
- (2) Owing to the quality control requirements flowing from the OPCW Laboratory, accreditation by the Dutch Accreditation Council (RvA), this activity fell outside the scope that can be audited because it was being performed outside the premises of OPCW Laboratory; and
- (3) The OPCW Laboratory had no control on this part of the process.

This consequently led to the development of procedures by the OPCW Laboratory to internalize the incorporation of electronic data into the OCAD. This has now been achieved for MS and GC(RI) data by the use of NIST (National Institute of Standards and Technology programs) and Microsoft Excel. (Commercial program provided by NIST (NIST MS Search/Analysis and AMDIS (Automated Mass Spectral Deconvolution and Identification System) for the management of the MS and GC(RI) data.) The procedure for the management of IR electronic data is still being developed; meanwhile, the IR part is still being used as hard copy (Portable Data Format (PDF)) version, as is the case with NMR. The hard copy is scanned into PDF and then managed using Adobe Acrobat. Adobe Acrobat is used for viewing the PDF files. Hyperlinks are used between the Excel spreadsheet and the PDF files for easy access. (This is a shortcut or jump that opens a

PDF document stored on the CD-ROM. Clicking the cell that contains the HYPERLINK function, activates Microsoft Excel (version 97 and above), to open the file stored at link_location.)

In the early days of the Secretariat, the OCAD was released and distributed as hard copies. The OCAD was distributed to Member States as a heap of paper, with data arranged according to the type of analytical technique. This was quite a cumbersome way to carry the OCAD and not user-friendly in its applications. Consequently, the Secretariat developed procedures that enable the final compilation of analytical data to be presented on CD-ROMs. The first release of the OCAD on a CD-ROM was the 'Central OPCW Analytical Database version 3' released in 2000. This was entirely a hard copy version, which was scanned into PDF. This was an improvement in that, instead of carrying a pile of paper, all data was now on a CD-ROM. However, it was still not user-friendly due to the fact that over 200 pages in PDF would be in one continuous file for each technique. The next step was to separate the PDF pages into single PDF pages that could be hyperlinked to the individual data entry listed in an excel spreadsheet. The next release (*PDF-OCAD v.4 and e-OCAD v.2*) contained the separated PDF files, which were linked to the index of the chemicals contained in the OCAD. For the first time, the electronic version of the OCAD was released containing electronic MS data only. The latest version of the OCAD on CD-ROM is the *PDF-OCAD v.5 and e-OCAD v.3* released October 2002 and the analytical data it contains was compiled entirely by the OPCW Laboratory.

4 THE SCHEDULES OF CHEMICALS

The schedule of chemicals (refer to **Chapter 2** for the list) is found in the Annex on Chemicals of the CWC. The schedule of chemicals categorizes the CWC-regulated chemicals into three schedules (1, 2, and 3) according to the chemical properties, toxicity, application, and level of regulation by the CWC. The number of analytical data that is in the version of the OCAD released in October 2002 is presented in Table 1.

The Table 1 illustrates the availability of data in the OCAD and its distribution according to the

Table 1. The distribution of the analytical data in PDF-OCAD v5 and e-OCAD v3 (CD-ROM release October 2002) according to the Schedules

Schedule number	Analytical data in PDF-OCAD v.5/e-OCAD v.3			
	IR	MS	NMR	GC(RI)
1.A.1	182	452	267	601
1.A.2	64	120	55	42
1.A.3	194	249	82	373
1.A.4	10	19	30	7
1.A.5	4	6	13	3
1.A.6	9	8	17	3
1.A.7	0	0	1	0
1.A.8	0	0	0	0
1.B.9	5	5	28	3
1.B.10	1	2	3	1
1.B.11	0	1	3	2
1.B.12	1	2	7	1
2.A.1	0	3	8	2
2.A.2	0	2	0	0
2.A.3	2	2	3	1
2.B.4	139	451	610	899
2.B.5	2	10	32	1
2.B.6	19	32	8	19
2.B.7	0	1	0	0
2.B.8	0	1	0	0
2.B.9	2	2	4	0
2.B.10	3	10	16	7
2.B.11	4	8	10	4
2.B.12	4	8	18	6
2.B.13	2	4	4	1
2.B.14	2	4	2	0
3.A.1	1	1	0	0
3.A.2	3	2	0	0
3.A.3	1	1	0	0
3.A.4	1	2	1	0
3.B.5	1	2	0	0
3.B.6	0	1	0	0
3.B.7	0	0	0	0
3.B.8	1	2	0	0
3.B.9	2	1	4	1
3.B.10	3	1	3	0
3.B.11	0	1	8	1
3.B.12	0	0	0	0
3.B.13	0	0	0	1
3.B.14	0	2	0	0
3.B.15	0	2	10	0
3.B.16	1	2	5	0
3.B.17	2	1	3	0
D.S.	0	27	0	18
Standards	0	34	0	8
S.NS	2	5	0	0
TOTAL	667	1489	1255	2005

schedules. The table clearly demonstrates that not all the analytical data is available for all the scheduled

compounds. The unavailable analytical data leaves gaps in the OCAD and it is important that these gaps are filled in the future, further extending the contents of the OCAD.

5 DESCRIPTION OF THE PROCESS

The mechanism of updating the OCAD ⁽²⁾ provides the regulatory framework for the processes of OCAD. The mechanism is consultative and enables approved validated spectra to be incorporated in the OCAD. The whole process entails sourcing the data, its validation by the VG, its approval by the Executive Council (EC), organization, management, distribution, and archiving of the OCAD.

This process starts with the Secretariat requesting Member States to contribute analytical data on a mutually agreed basis in the most cost-efficient way. More than 90 % of the analytical data in OCAD has been contributed free of charge to the Secretariat. These analytical data are validated before they are proposed for incorporation into the OCAD. Once a batch of analytical data has been technically validated, approval is obtained from the Member States for the incorporation of these spectra into the OCAD.

The Director-General presents a list of newly validated analytical data to the EC for approval. The list is also announced to all Member States 30 days in advance of the Council session in order to give them an opportunity to communicate to the Council any concerns that they may have in relation to the inclusion of the proposed analytical data. The Director-General requests the EC to approve the inclusion of analytical data into the OCAD. In the event that the EC decide not to approve individual analytical data presented to it, the Director-General may undertake to provide further information to the EC addressing the concerns expressed.

The overview of the process of updating the OCAD, including its management, is schematically represented in Figure 2.

6 VALIDATION GROUP

The VG was established in 1997 and is responsible for the technical validity of the analytical data in

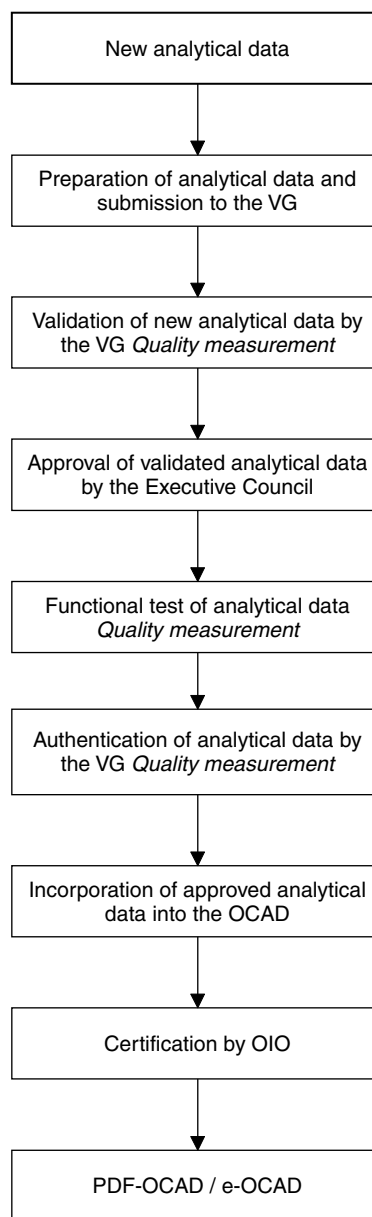


Figure 2. The process of the OPCW Central Analytical Database

the OCAD. The respective Governments of the States Parties nominate the members of the VG and the Secretariat approves the nominations. The VG appoints a Chairman. The group has four subgroups divided into four different analytical techniques. A Coordinator heads each subgroup. The subgroups are

referred to as the MS, IR, NMR, and GC Retention Indices subgroups.

7 VALIDATION AND APPROVAL OF ANALYTICAL DATA

The types of analytical data contributed are: Mass Spectra (MS), Infrared (IR) spectra, Nuclear Magnetic Resonance (NMR) spectra and Gas Chromatography Retention Indices (GC(RI)). The analytical data are contributed either in hard copy or as electronic files in acceptable formats. MS data is contributed in the electronic format.

The original analytical data submitted by Member States to the OPCW Laboratory are archived. The original analytical data is assigned unique OPCW codes at the OPCW Laboratory before archiving. This code comprises the identification number of the laboratory that contributed data, a number that corresponds to the type of analytical technique, a number corresponding to the data entry into the OCAD, and letters that distinguish vapor phase IR (v) from condensed phase and other types of IR spectra, diastereoisomers (a, b, c, etc.) and resubmitted data (r, s, t, etc.) (see Figure 3).

The information related to the new analytical data is registered in a control register, which is an Excel spreadsheet. The control register is updated each time new or resubmitted analytical data is received at the OPCW Laboratory, or when there is a change in the data status.

The new analytical data is checked against specific requirements of the analytical technique. The analytical data must be in the agreed format and accompanied by essential information on the chemical and the recording conditions ⁽⁴⁾. The hard copy version of the OCAD is scanned into PDF for validation and

further processing for final presentation on a CD-ROM. Minor errors in the analytical data maybe corrected on the PDF copy. These errors, which are mainly reported during the validation process, are corrected by editing the PDF file using Adobe Acrobat software. In cases when the errors are major, data is requested for resubmission.

The new analytical data is validated on the basis of the electronic version of the data as the true master copy. In the past, the hard copy used to be the only master copy for all techniques. This new approach applies only to the data that can be managed by electronic means. So far this approach has been successfully applied to the validation of MS technique only. The main reason for that is the availability of the software for managing the MS data (NIST MS Search). In future, procedures shall be developed for the other analytical techniques, for example IR, to be based on the electronic version as the true master copy.

To facilitate the validation process on the basis of the above approach, the MS electronic data is contributed to the OPCW Laboratory in any of the following electronic formats JCAMP, NIST ASCII, AMDIS, and NIST MS Database. Contributing laboratories provide mostly the NIST MS Database format, with structures. The OPCW Code and the Schedule number are placed in the synonym field of the database. The file is submitted to the OPCW Laboratory either as the NIST MS User Database or the corresponding set of text files representing the MSP (Spectral) and MOL (Structure) information. The NIST MS Search/Analysis programs are used for the management of the MS electronic data and also GC(RI) once merged with the MS data.

In the case where MS analytical data has been contributed in the other formats, it is prepared into a NIST MS Database, using the NIST MS Search program. This program is used to create MS spectral files from the following electronic formats: JCAMP, NIST ASCII, AMDIS, and NIST MS Database. If the analytical data contains no chemical structure, the structure is created in ISIS Draw and then imported into the database as an MOL file. The information associated with the MS analytical data is handled by the NIST program.

The new analytical data is validated by the VG. It is approved by the EC. The coordinators of each analytical technique and the chairman of the VG

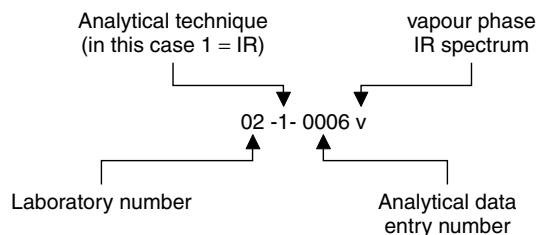


Figure 3. Example of the OPCW Code: 02-1-0006v

then authenticate the approved data. The purpose of authentication is to ensure that the analytical data for incorporation into the OCAD corresponds to the analytical data that was validated by the VG and to ensure that any corrections noted by the VG have been made. The minor corrections and corrected analytical data are sent to the Chairman of the VG for authentication. The process involves the verification of the authenticity of the final PDF files and electronic databases to be incorporated into the OCAD.

After the authentication, the Secretariat's Office of the Internal Oversight (OIO) then certifies this data. The OPCW Laboratory has a quality system, which has been accredited by the Dutch Accreditation Council (RvA). The accreditation covers the quality system as well as the specific activity of the OCAD process and other processes. The OPCW Laboratory has in place quality documents (standard operating procedures (SOPs) and work instruction) for the OCAD process.

8 PREPARATION OF THE NEW VERSION OF THE OCAD

The new analytical data approved by the EC is incorporated into the existing OCAD. The existing version of OCAD is updated by adding the PDF and electronic files of the new analytical data to the PDF and electronic files of the existing version.

The new version of the OCAD also includes the corrections made to the contents of the existing version of the OCAD. Finally, the new compilation

of the OCAD (PDF-OCAD/e-OCAD) is assembled on a CD-ROM for issuance to the Member States and also for the preparation of on-site databases.

9 PRESENTATION OF DATA ON CD-ROM

The final compilation of the OCAD to be included on the CD-ROM is in two forms, namely, hard copy (scanned into PDF) and electronic version (NIST MS Database). The electronic version of the database is the digital form of the data corresponding to the hard copy of MS and IR data. The GC(RI) data is part of the electronic NIST MS database.

The final PDF files of all the four analytical techniques, the NIST MS Database (MS only), the excel spreadsheet that indexes all the data (PDFIndex) that is on the CD-ROM, and the HTML browser are all included on the CD-ROM. The certificates from OIO are also included on the CD-ROM as PDF. The PDFIndex, NIST MS Database, and other utilities are hyperlinked to the HTML page. The analytical data presented in the excel spreadsheet (PDFIndex) are all hyperlinked to the individual PDF files and electronic MS files (refer to Table 2). The MS data in the NIST MS Database is hyperlinked via NIST links. All these files are put together and written onto a CD-ROM. The resulting CD-ROM is self-installing and automatically launches an HTML browser that allows the user to access analytical data and information on the CD-ROM from the browser.

Table 2. An extract from the MS ANALYTICAL DATA spreadsheet PDFIndex showing the OPCW Code, Chemical name, CAS number, Schedule number, Link to PDF files and link to the electronic MS data in the NIST MS Database

OPCW code	Chemical name	CAS	Schedule	Link to PDF file	Link to NIST MS search
01-2-0001	Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate	77-81-6	1.A.02	PDF	NIST
01-2-0002	Isopropyl <i>N,N</i> -dimethylphosphoramidocyanidate	63815-55-4	1.A.02	PDF	NIST
01-2-0003	Propyl <i>N,N</i> -dimethylphosphoramidocyanidate	162085-86-1	1.A.02	PDF	NIST
01-2-0004	Diethyl <i>N,N</i> -dimethylphosphoramidate	2404-03-7	2.B.06	PDF	NIST
01-2-0005	1-Methylpentyl methylphosphonofluoridate	13172-12-8	1.A.01	PDF	NIST
01-2-0006	1,3-Dimethylbutyl methylphosphonofluoridate	352-53-4	1.A.01	PDF	NIST
01-2-0007	Propyl methylphosphonofluoridate	763-14-4	1.A.01	PDF	NIST
01-2-0008	Cyclohexylmethyl methylphosphonofluoridate		1.A.01	PDF	NIST
01-2-0009	Isopropyl methylphosphonofluoridate	107-44-8	1.A.01	PDF	NIST
01-2-0011	Pentyl methylphosphonofluoridate	13454-59-6	1.A.01	PDF	NIST

10 PREPARATION OF ON-SITE DATABASES

The on-site databases are prepared at the OPCW Laboratory for Verification activities. Two types of analytical data for on-site analytical databases are provided for an on-site inspection:

- (a) electronic MS analytical data and GC retention indices (in AMDIS) for use with the on-site GC/MS instruments; and
- (b) individual hard copies (print-outs from PDF) of analytical data for specific compounds on any analytical technique.

All the types of analytical data can be provided in hard copy if so required. At the moment, only MS data can be provided in electronic format as a searchable on-site analytical database to conduct on-site analysis.

Preparation of the analytical data in hard copy entails printing of the PDF files from the OCAD and certification for on-site use. The different types of the analytical data are prepared separately and each technique is certified separately. The analytical data requested is extracted from the OCAD and then certified for on-site use. The lifetime of this analytical data is the duration of the concerned on-site inspection.

The electronic MS on-site databases are prepared from the certified electronic version of the OCAD (e-OCAD). This version is compiled using the NIST programs (NIST MS Search/Analysis and AMDIS) and is in the form of a NIST MS Database. The e-OCAD does not contain Gas Chromatography retention indices (GC(RI)) that are required for on-site use. To create an electronic MS on-site database, the certified version of e-OCAD, which at the moment contains only MS data, is merged with the certified (GC(RI)), which is in an excel spreadsheet. After merging these data, an AMDIS version of the database is created, which is compatible with the AMDIS software used by the on-site GC/MS equipment (for more details on the software, refer to **Chapter 2** and **Chapter 4**)⁽⁵⁾. The MS on-site database can be sent for an inspection as a whole database or as an extract of the whole database. AMDIS is software that is used by the GC/MS on-site equipment

for postprocessing of the measured mass spectral data.

11 FUNCTIONAL TEST OF THE ELECTRONIC VERSION OF THE OCAD

The OPCW Laboratory performs functional tests on the authenticated, certified, and on-site analytical databases. These are tests that verify that the analytical database is functional. So far, this procedure is fully developed for the MS analytical databases. The electronic version of OCAD (which so far consists of MS analytical data only) is tested with the NIST MS Search/Analysis program. The on-site MS analytical databases are tested using AMDIS software. A NIST or AMDIS library is created and this becomes the target library for a library search. A search is performed on a newly measured MS spectrum (raw data files) containing a number of chemicals that are present in the target library. Identification of the targeted spectrum indicates that the created library is functional.

12 THE DEVELOPMENT OF THE OCAD

In the last five years, the OCAD has grown at a steady pace as shown in Figures 4 and 5. In particular, the MS and GC(RI) data have increased at a steady rate and are expected to continue in this way. The NMR and IR data, on the other hand, have not been growing at the same rate as the MS and GC(RI), and appear to be leveling off. The main reason for this is that the Secretariat has to date been focusing on MS and GC(RI) data for its portable GC/MS inspection equipment. A second equally important factor may be that the MS and GC(RI) data have been obtained by quick microsynthesis often producing mixtures of chemicals, while recording of NMR data would require rather pure compound, and therefore, need additional cleanup procedures.

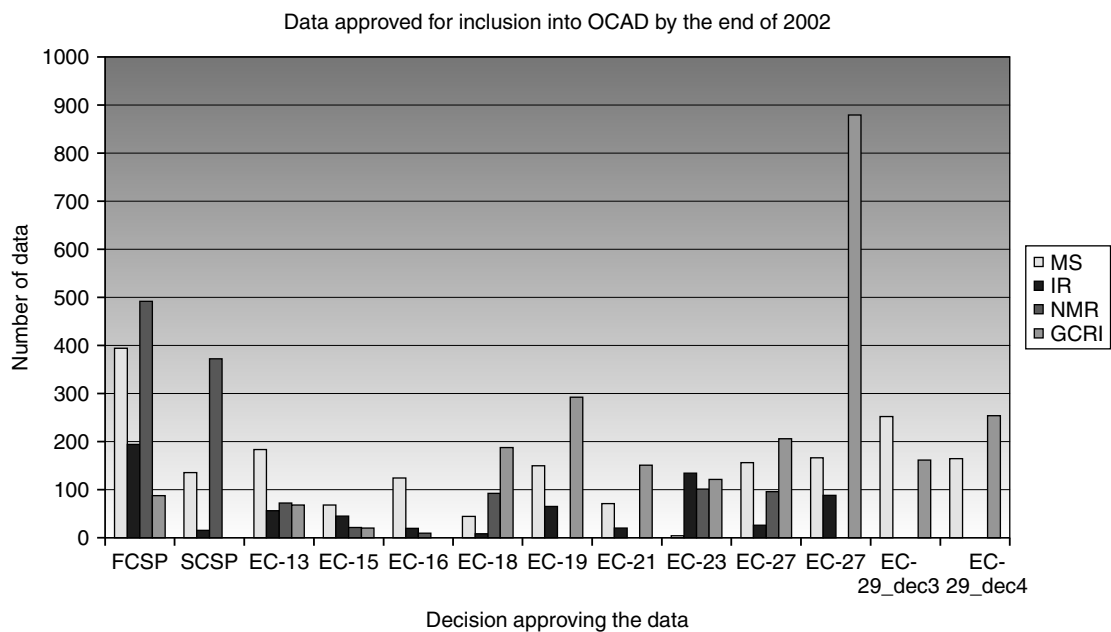


Figure 4. This illustrates the variation of the amount of analytical data approved by the Conference of States Parties (CSP) and the Executive Council (EC) 1997–2002

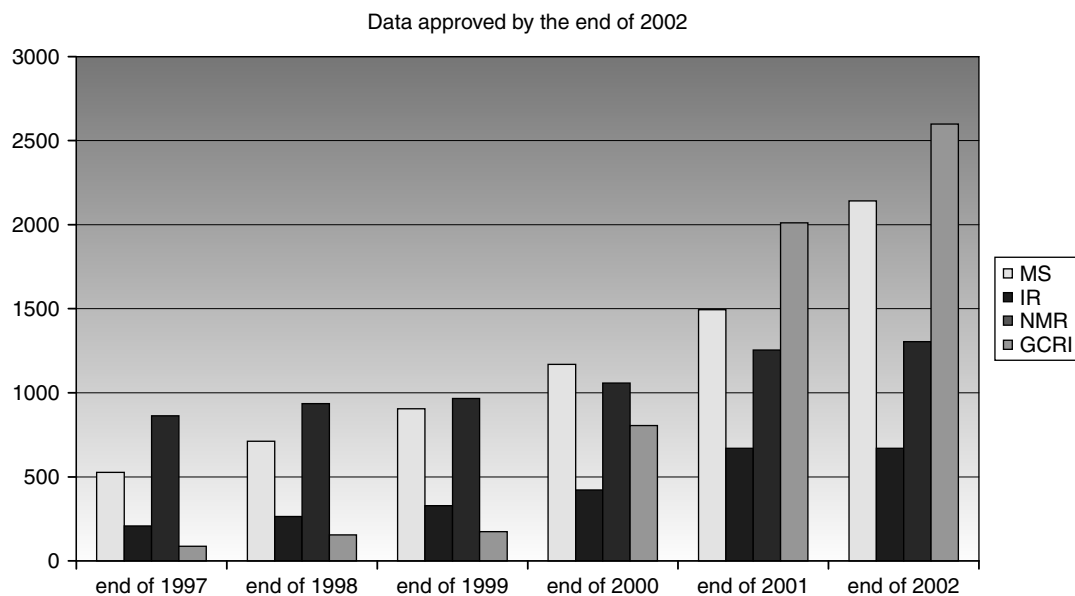


Figure 5. This illustrates the growth of the OCAD 1997–2002. The amount of data is cumulative over time, with the amount indicated at the end of 2002 being the total amount of data approved for inclusion since the conception of the OCAD

13 DESCRIPTION OF THE OCAD CONTENT

On the basis of the CD-ROM released in October 2002, PDF-OCAD v.5 and e-OCAD v.3, the CD-ROM contains a total number of 5419 analytical data measured from 1707 chemicals. All this data is managed by an Excel spreadsheet (refer to Table 2 for an example of the spreadsheet). The number of analytical data and chemicals on the CD-ROM in relation to each technique is illustrated in Table 3 and Figure 6

14 RULES FOR NAMING CHEMICALS IN THE OPCW CENTRAL ANALYTICAL DATABASE

The VG, in cooperation with the Secretariat, established rules for naming chemicals in the OCAD. These rules in general are based on the names given in the CWC (Annex on Chemicals). In cases where the guidance from the CWC is insufficient to provide an unambiguous chemical name, additional rules have been developed to assist (Annex 1). These rules have an explicit relationship to the structure of the compound, which enables a user to identify

Table 3. The number of analytical data and chemicals in the OCAD (PDF-OCAD v.5 and e-OCAD v.3)

Analytical technique	Number of analytical data	Number of chemicals
IR	667	520
MS	1490	1081
NMR	1255	265
GC(RI)	2007	1238

the compound from the name. Along with the rules, examples have been given with the systematic names for each schedule group. These rules have already been implemented; however, the list of examples continues to grow as new cases arise, which means that the rules are continuously revised.

These rules are based on the IUPAC (International Union of Pure and Applied Chemistry) nomenclature, but largely adapted to suit the specific cases of the chemicals in the Schedules. The rules allow only one name for each compound; in this way, the name becomes a unique identifier for the compound. This facilitates the management of the OCAD.

Laboratories submitting new data are requested to follow these naming rules. These rules are published by the Secretariat to all the States Parties' laboratories that submit new analytical data. When the chemical names of new submitted data do not conform

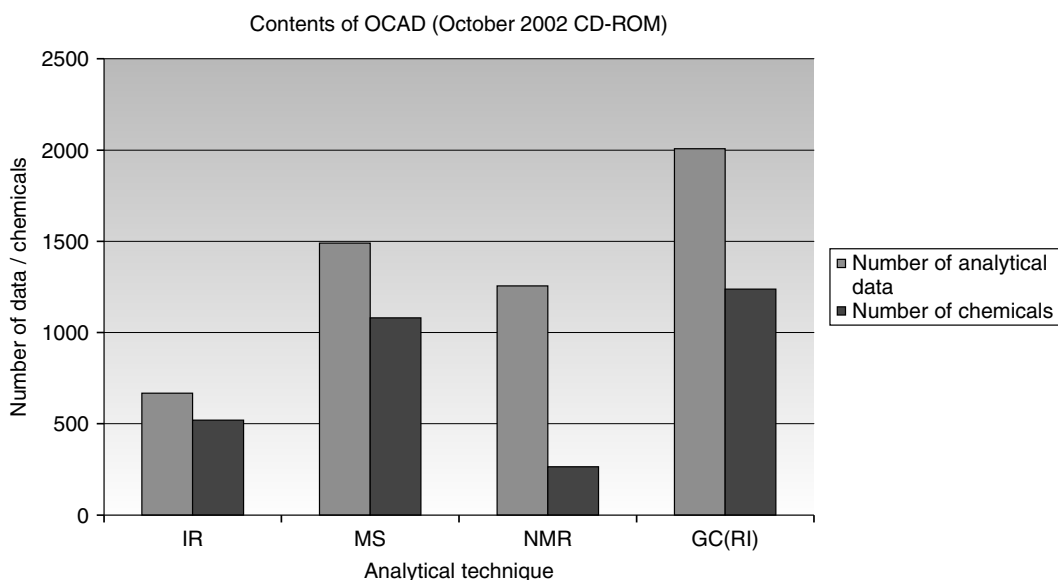


Figure 6. The graph illustrates the content of the OCAD in terms of the analytical data and the chemicals represented

to the naming rules, then the VG or the OPCW Laboratory corrects them.

The OPCW Laboratory prepares on-site analytical databases for use during an inspection, and the success of the process of creating the MS on-site database depends on the correctness and consistency of the chemical name.

15 THE TYPE OF ANALYTICAL DATA IN OCAD

The analytical data (spectra) for each chemical available in the OCAD for each technique varies from single to multiple entries. It is quite common to find at least three MS spectra for particular widely known scheduled compounds. This has several advantages, for instance, if on-site data is requested, there is a range of choice of data to choose from. Spectra acquired from different instruments or conditions can be selected to suit the scenario of an inspection. As the database grows, however, analytical data determined to be redundant or of low quality maybe replaced.

The NMR data that is in OCAD is one-dimensional NMR spectra measured on FT (Fourier Transform) spectrometers with a proton frequency of 200 MHz or higher. The types of spectra are ^1H spectra; ^{13}C spectra (coupled and ^1H -decoupled); ^{31}P spectra (coupled and ^1H -decoupled); and ^{19}F spectra. It follows therefore that the nuclei present in the OCAD are ^1H , ^{31}P , $^{31}\text{P}\{^1\text{H}\}$, ^{19}F , ^{13}C , and $^{13}\text{C}\{^1\text{H}\}$.

These NMR spectra are used like a fingerprint to identify a chemical by comparison with its reference spectrum recorded from the authentic chemical under comparable conditions ⁽⁶⁾. In proficiency tests, NMR is a very useful complementary technique to MS and IR. The limitation is that NMR data is only available in the OCAD as hard copy (PDF). This means that a searchable electronic database that can be used as a library in an instrument is not available.

The MS analytical data available in the OCAD is Electron Impact/Mass Spectra (EI/MS) ⁽⁷⁾. The incorporation of chemical ionization (CI) mass spectra, MS/MS data, and data from other MS ionization techniques was previously considered. The VG came to the conclusion that, whilst these techniques had valuable information for the

identification of scheduled compounds, the data produced are highly variable. The mass spectra that are submitted for possible inclusion in the OCAD must meet specific criteria to facilitate evaluation by the VG. Since the MS data is distributed together with a demo version of the NIST programs (comprising of NIST MS Search program and AMDIS), the format to submit this MS data is as a NIST MS Database (*NIST MS User Database or the corresponding set of text files representing the MSP (Spectral) and MOL (Structure) information* (more information on the NIST MS database and AMDIS can be obtained from NIST). The advantages of this are that the data is contributed with structures and in a usable format. If this is not possible, other formats are acceptable, and these are JCAMP and NIST, ASCII.

The IR analytical data in the OCAD has a minimum resolution of 4 cm^{-1} for condensed phase and normal gas phase spectra, and 8 cm^{-1} for GC light-pipe spectra. The minimum spectral range is at least $3700\text{--}700\text{ cm}^{-1}$ for condensed phase and $3700\text{--}750\text{ cm}^{-1}$ for normal gas phase spectra. The reference IR spectra in the OCAD can be used for the identification of chemicals from CWC related samples. However, when used in this way, it is important to support the identification with other analytical techniques, like GC/MS ⁽⁸⁾.

The GC retention index data in the OCAD is measured under the same conditions as the GC conditions of the GC/MS spectra in the OCAD. These conditions are as follows:

- Column: length 30 m, internal diameter 0.25 mm, film thickness $0.25\text{ }\mu\text{m}$
- Stationary phase: 95 % dimethyl, 5 % phenyl-siloxane
- Temperature programme: $40\text{ }^\circ\text{C}$ (2 min)– $10\text{ }^\circ\text{C/}$ min– $280\text{ }^\circ\text{C}$ (10 min).

The GC retention index data must be supported by additional determinations such as an independent measurement or extrapolation from a series of homologous chemicals where applicable. The identification of chemicals from CWC-related samples must be supported by other data, for example, GC/MS.

16 THE USE OF THE OCAD

1. The Verification related activities by the Secretariat

(a) On-site inspection

The OPCW carries out on-site inspections in accordance with the requirements of the CWC. The OCAD has been used successfully in inspection scenarios as referred to in Table 4 and Figure 7 in the period from 1998 to 2002.

Table 4. Use of the OCAD and the types of inspection scenarios experienced by the end of 2002

Inspection type	Number of inspections
ACW (Abandoned chemical weapons)	3
CHAL-EX (Challenge inspection exercise)	5
CWDF (Chemical weapons destruction facility)	19
DHCW (Destruction of hazardous chemical weapons)	2
AU-ex (Alleged use exercise)	2
Sched1 (Schedule 1)	4
Sched2 (Schedule 2)	1
Sched3 (Schedule 3)	1
OCPPF (Other chemical production facilities)	3

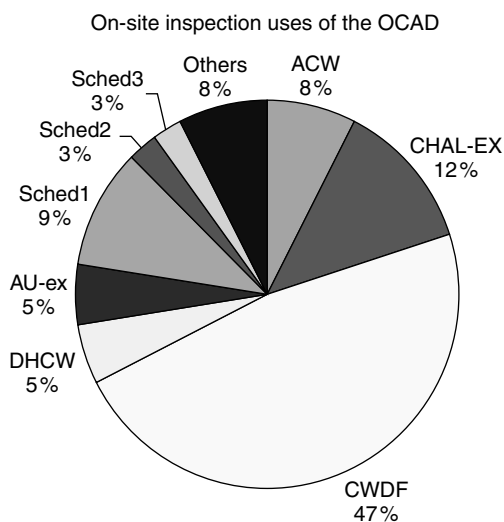


Figure 7. Usage of the OCAD in the cited Verification activities

It is important to note that the above distribution of the uses of the OCAD is anticipated to change depending on the changing priorities of the Organization with regards to these activities.

(b) Testing and calibration of analytical equipment

Analytical equipment is tested and calibrated for use on site (e.g. Calibration of the portable GC/MS instrument used for on-site inspections). The OPCW Laboratory tests and calibrates the portable GC/MS instrument in preparation for on-site inspection use. In the process, raw MS analytical data is acquired and the tests and calibration results are checked against the target library of MS analytical database created from the OCAD.

2. Distribution to SP or use by SPs

(a) Proficiency Tests

The OPCW Laboratory conducts a Proficiency Testing program (as described in **Chapter 6**) for the purpose of selecting and maintaining designated laboratories. The OCAD has been used extensively by all laboratories participating in the tests and will undoubtedly be an important reference in any event of off-site analysis.

(b) Use by the States Parties laboratories

The OCAD presented and released on a CD-ROM is distributed to States Parties for use by their own laboratories and for the purposes of supporting the CWC. OCAD is becoming the source of analytical data and related information on chemicals related to the CWC.

17 FUTURE DEVELOPMENTS

Extending the contents of the OCAD (immediate future) When conducting on-site analysis, the OCAD is the only source of reference, in particular, if the instrument is operated in 'blinded mode' (refer to **Chapter 2** and **Chapter 4**). It is therefore important to ensure that the OCAD is as extensive as possible so as to cover every likely scenario. The States Parties and the Secretariat continue to work on expanding the OCAD by including data on chemicals relevant to the CWC, in order to cover as many of these chemicals as possible.

Expanding the scope of OCAD (long term) The scope of the analytical data in OCAD is limited to Schedule chemicals and certain derivatives. The analytical data of certain unscheduled chemicals that are either related to the scheduled chemicals (e.g. well-known impurities, additives, and degradation products) or chemicals that are a cause for concern, given their potential as chemical warfare agents but which are not scheduled (SAB), are not included in the OCAD. Nonscheduled degradation products of scheduled chemicals, riot control agents, and derivatives of scheduled chemicals other than those mentioned above are also not included. Efforts are underway to extend the scope of the OCAD to include the data of these chemicals.

Development of the validation process of IR data based on the electronic version as the master Experience of the MS data so far has shown that validation of data on the basis of the electronic version is much easier and more practical. Efforts are underway to identify the software that can be used to manage the electronic IR data in order to facilitate its validation on the basis of the electronic version.

CD-ROM As part of the continual development of the ease of use of the OCAD and its presentation on CD-ROM, a new browser based on HTML, to replace the Excel spreadsheet (PDFIndex), is currently under consideration by the Information Systems Branch of the Secretariat. Software identification and development that can improve the CD-ROM will continue to be explored.

ABBREVIATIONS AND ACRONYMS

$^{13}\text{C}^1\text{H}$ Experiment: Carbon-13
Observation With Simultaneous
 ^1H Decoupling

^{19}F Nucleus: Fluorine-19; Experiment:
Fluorine-19 Observation

^1H Nucleus: Hydrogen-1 or Proton;
Experiment: Hydrogen-1 or Proton
Observation

$^{31}\text{P}^1\text{H}$ Experiment: Phosphorus-31
Observation With Simultaneous
 ^1H Decoupling

^{31}P Nucleus: Phosphorus-31;
Experiment: Phosphorus-31
Observation

ACW Abandoned Chemical Weapons

AMDIS Automated Mass Spectral
Deconvolution and Identification
System

CAS Chemical Abstracts Service

CHAL-EX Challenge Inspection Exercise

CI Chemical Ionization

CSP Conference of States Parties

CWC Chemical Weapons Convention

CWDF Chemical Weapons Destruction
Facility

DHCW Destruction of Hazardous
Chemical Weapons

EC Executive Council

EI/MS Electron Impact/Mass Spectra

EIF Entry into Force

FT Fourier Transform

GC/MS Gas Chromatography/Mass
Spectrometry

GC(RI) Gas Chromatography Retention
Indices

IR Infrared

IUPAC International Union of Pure
and Applied Chemistry

MS Mass Spectra

NIST National Institute of Standards
and Technology

NMR Nuclear Magnetic Resonance

OCAD OPCW Central Analytical
Database

OCPF Other Chemical Production
Facilities

OIO Office of the Internal
Oversight

OPCW Organization for Prohibition
of the Chemical Weapons

PC Preparatory Commission

PDF Portable Data Format

SAB Scientific Advisory Board

SOPs Standard Operating Procedures

SPs States Parties
VG Validation Group

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ANNEX 1

Rules for naming compounds in the OPCW analytical database

1. In general, the name (spelling, punctuation, spaces, etc.) should be based on the name given in the Convention (Annex on Chemicals).
2. Additional rules provide directions for cases when information in the Schedules of Chemicals is insufficient to designate the name.
 - 2.1 First letter of the name in capitals (only exception the structural and stereodescriptors sec-, tert-, cis- and trans-).
 - 2.2 Use the trivial names for the following radicals:
Saturated branched: Isopropyl, Isobutyl, sec-Butyl, tert-Butyl. Also use pinacolyl instead of 1,2,2-trimethylpropyl. However, pinacolyl alcohol should preferably be named 3,3-dimethyl-2-butanol.
Unsaturated: Vinyl, Allyl, Isopropenyl.
 - 2.3 When a compound has several substituents, list the substituents in alphabetical order disregarding the presence of N, O or S prefixes and the descriptors sec-, tert-, cis- and trans-; but see rule 2.5 below.
 - 2.4 The radicals isobutyl, isopropenyl and isopropyl are considered as one entity and are listed in alphabetical order starting from 'iso'.
 - 2.5 The substituents in Schedule 1.A.03 and 1.B.10 compounds are listed in the order 'alkyl 2-dialkylaminoethyl', in line with the names given in the Convention, but constituting an exemption to rule 2.3. The same exemption applies to 2.B.4 compounds containing the 'alkyl 2-dialkylaminoethyl' moieties.
 - 2.6 Parentheses are placed around prefixes defining substituted substituents and after the numerical multiplicative prefixes

- (bis-, tris-, etc.), around simple substituent prefixes to separate locants of the same type referring to different structural elements, and to avoid ambiguity.
- 2.7 For radicals with a branching structure, the name should be derived from the longest continuous chain starting (position 1) at the conjunction with the parent structure. Examples:
- the methylphosphonofluoridate made using 5-methyl-3-hexanol is 1-Ethyl-3-methylbutyl methylphosphonofluoridate.
 - the name 1-ethyl-2-methylpropyl should be used instead of 1-isopropylpropyl.
- 2.8 Differentiation is made between thiolate and thionate depending on whether the S-atom is single or double bonded to the phosphorous atom.
- 2.9 The name should be as short as possible and unnecessary characters should be left out:
- the *n*- in *n*-alkyl;
 - the 1- before 1-alkyl in case of a normal alkyl chain;
 - the *O* in *O*-Alkyl alkylphosphonohalidates;
 - the *O* in *O*-Alkyl *S*-2-dialkylaminoethyl alkylphosphonothiolates belonging to Schedule 1.A.03; and
 - unnecessary brackets and parenthesis.
- 2.10 Hydrochloride salts of scheduled 2.B.10, 2.B.11 and 2.B.12 chemicals should be named as the free amines with the addition of hydrochloride.
3. Applications of those rules are illustrated by examples for scheduled compounds and derivatives associated with the Scheduled compounds.

Examples of names of Schedule compounds

Schedule	Name
1.A.01	Alkyl alkylphosphonofluoridate
1.A.02	Alkyl <i>N,N</i> -dialkylphosphoramidocyanidate
1.A.03	Alkyl <i>S</i> -2-dialkylaminoethyl alkylphosphonothiolate
1.A.04	2-Chloroethylchloromethylsulfide Bis(2-chloroethyl)sulfide Bis(2-chloroethylthio)methane 1,2-Bis(2-chloroethylthio)ethane 1,3-Bis(2-chloroethylthio)propane 1,4-Bis(2-chloroethylthio)butane 1,5-Bis(2-chloroethylthio)pentane Bis(2-chloroethylthiomethyl)ether Bis(2-chloroethylthioethyl)ether
1.A.05	2-Chlorovinylchloroarsine Bis(2-chlorovinyl)chloroarsine Tris(2-chlorovinyl)arsine
1.A.06	Bis(2-chloroethyl)ethylamine Bis(2-chloroethyl)methylamine Tris(2-chloroethyl)amine
1.A.07	Saxitoxin
1.A.08	Ricin
1.B.09	Alkylphosphonic difluoride
1.B.10	Alkyl 2-dialkylaminoethyl alkylphosphonite
1.B.11	Isopropyl methylphosphonochloridate
1.B.12	Pinacolyl methylphosphonochloridate
2.A.01	<i>O,O</i> -Diethyl <i>S</i> -2-diethylaminoethyl phosphorothiolate
2.A.02	1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene

(continued overleaf)

(continued)

Schedule	Name
2.A.03	3-Quinuclidinyl benzilate
2.B.04	To avoid any confusion the O and S groups should be indicated in esters, when sulfur is present. Examples: Methylphosphonothioic acid $[(\text{CH}_3\text{P}(=\text{S})(\text{OH})_2]$ <i>O</i> -Ethyl methylphosphonothionate $[(\text{C}_2\text{H}_5\text{O})\text{P}(=\text{S})(\text{CH}_3)(\text{OH})]$ <i>O,O</i> -Diethyl methylphosphonothionate $[(\text{C}_2\text{H}_5\text{O})_2\text{P}(=\text{S})(\text{CH}_3)]$ <i>O</i> -Propyl <i>O</i> -trimethylsilyl propylphosphonothionate <i>O</i> -Ethyl <i>S</i> -ethyl methylphosphonothiolate $[(\text{C}_2\text{H}_5\text{O})\text{P}(=\text{O})(\text{CH}_3)(\text{SC}_2\text{H}_5)]$ <i>S</i> -Ethyl <i>O</i> -methyl methylphosphonothiolate <i>O</i> -Ethyl <i>S</i> -2-methylthioethyl methylphosphonothiolate <i>O</i> -Ethyl <i>S</i> -ethyl methylphosphonothiolothionate $[(\text{C}_2\text{H}_5\text{O})\text{P}(=\text{S})(\text{CH}_3)(\text{SC}_2\text{H}_5)]$ <i>O</i> -Ethyl methylphosphonothionochloridate $[(\text{C}_2\text{H}_5\text{O})\text{P}(=\text{S})(\text{CH}_3)(\text{Cl})]$ Methylphosphonous dichloride $(\text{CH}_3\text{P}-\text{Cl}_2)$ Methylphosphonic dichloride $[(\text{CH}_3\text{P}(=\text{O})-\text{Cl}_2)]$ Methylphosphonothioic dichloride $[(\text{CH}_3\text{P}(=\text{S})-\text{Cl}_2)]$ Dimethyl methylphosphonate Bis(1,2-dimethylpropyl) methylphosphonate Benzyl 1,2-dimethylpropyl ethylphosphonate Methyl methylphosphonate instead of methyl methylphosphonic acid Methylphosphonic acid Isobutyl methylphosphonochloridate Isopropyl methylphosphonoazidate 2-Diisopropylaminoethyl methylphosphinate $[(i-\text{C}_3\text{H}_7)_2\text{N}-\text{CH}_2\text{CH}_2-\text{O}-\text{P}(=\text{O})(\text{H})(\text{CH}_3)]$ Methyl 2-diethylaminoethyl methylphosphonate <i>O</i> -Ethyl <i>S</i> -2-dibutylaminoethyl methylphosphonothiolate Bis(<i>S</i> -2-diethylaminoethyl) methylphosphonodithiolate <i>O</i> -Ethyl <i>S</i> -3-dimethylaminopropyl methylphosphonothiolate Diethyl methylphosphonite $[(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{CH}_3)]$ Dicyclohexyl dimethylpyrophosphonate $[(\text{C}_6\text{H}_{11}\text{O})(\text{CH}_3)\text{P}(=\text{O})-\text{O}-\text{P}(=\text{O})(\text{CH}_3)(\text{C}_6\text{H}_{11}\text{O})]$ Dicyclohexyl dimethylpyrophosphonodithionate $[(\text{C}_6\text{H}_{11}\text{O})(\text{CH}_3)\text{P}(=\text{S})-\text{O}-\text{P}(=\text{S})(\text{CH}_3)(\text{C}_6\text{H}_{11}\text{O})]$
2.B.05	<i>N,N</i> -Dialkylphosphoramidic dihalide
2.B.06	Dialkyl <i>N,N</i> -dialkylphosphoramidate Dimethyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidate
2.B.07	Arsenic trichloride
2.B.08	2,2-Diphenyl-2-hydroxyacetic acid
2.B.09	3-Quinuclidinol
2.B.10	2- <i>N,N</i> -Dialkylaminoethyl chloride <i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethyl chloride
2.B.11	2- <i>N,N</i> -Dialkylaminoethanol <i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethanol
2.B.12	2- <i>N,N</i> -Dialkylaminoethanethiol <i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethanethiol
2.B.13	Bis(2-hydroxyethyl)sulfide
2.B.14	3,3-Dimethyl-2-butanol
3.A.01	Carbonyl dichloride
3.A.02	Cyanogen chloride
3.A.03	Hydrogen cyanide
3.A.04	Trichloronitromethane
3.B.05	Phosphorous oxychloride
3.B.06	Phosphorous trichloride
3.B.07	Phosphorous pentachloride
3.A.08	Trimethyl phosphate
3.A.09	Triethyl phosphate
3.A.10	Dimethyl phosphate
3.A.11	Diethyl phosphate

(continued)

Schedule	Name
3.B.12	Sulfur monochloride
3.B.13	Sulfur dichloride
3.B.14	Thionyl chloride
3.B.15	Ethyl-diethanolamine
3.B.16	Methyl-diethanolamine
3.B.17	Triethanolamine

Example of names for derivatives (D.S.)

D.S.	Type of name
1.A.05	2-(2-Chlorovinyl)-5-methyl-1,3,2-benzodithiarsole
2.B.08	Bis(trimethylsilyl)benzilate
2.B.09	3-Quinuclidinyl trimethylsilyl ether
2.B.07	2-Chloro-5-methyl-1,3,2-benzodithiarsole
2.B.11	2- <i>N,N</i> -Dialkylaminoethyl trimethylsilyl ether
	2- <i>N,N</i> -Dialkylaminoethyl tert-butyldimethylsilyl ether
2.B.12	2- <i>N,N</i> -Dialkylaminoethyl trimethylsilyl sulfide
2.B.13	Bis(2-trimethylsilyloxyethyl)sulfide
3.B.15	Bis(2-trimethylsilyloxyethyl)ethylamine
3.B.16	Bis(2-trimethylsilyloxyethyl)methylamine
3.B.17	Tris(2-trimethylsilyloxyethyl)amine
	Tris(2-tert-butyldimethylsilyloxyethyl)amine

CHAPTER 8

Analysis Strategy for Analysis of Chemicals Related to the Chemical Weapons Convention in an Off-site Laboratory

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1 INTRODUCTION

Seventeen States Parties (SP) have volunteered to provide services of their high-level chemical analytical laboratories to the Organization for prohibition of the Chemical Weapons (OPCW). From these SPs, 18 laboratories (2004) have been designated by the Director-General (DG) of the OPCW to perform analyses as part of the implementation of the Chemical Weapons Convention (CWC) ⁽¹⁾. For the purpose of effective implementation, for example, analysis of samples taken during inspections or

challenge inspections, even more designated laboratories (DLs) may be needed especially in geographical areas with no DL.

Before the designation, the laboratories developed their laboratory methods for testing, constructed and made operational their laboratory quality system and obtained its accreditation, and continued the participation and successful performance in the OPCW proficiency tests (PT). Analytical methods in particular and a certain level of quality assurance systems have existed in the laboratories, involved often in research, well before their designation. Participation in the international interlaboratory

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comparison tests has given the laboratories an opportunity to further develop their analytical methods and quality systems. Also, the OPCW has had the opportunity to develop procedures for conducting the PTs during the eight years of testing and with experience of 16 completed tests.

Through continued participation and success in PTs the DLs have reached an acknowledged level in testing. While the laboratories maintain their readiness to testing, they also continue research and methods development. Many of the DLs participate in training, seminars, and workshops, not only to increase the competence of own laboratory personnel, but also of other SP laboratories. These kinds of activities serve in skills enhancement and capacity building worldwide.

The OPCW and the DL has a client–service provider relationship. The DL operates its quality system according to an international standard and by doing so, it assures the client of its reliability in testing and of full compliance in confidentiality matters. The DL cannot afford for mistakes in its services. On the other hand, the OPCW uses the services of the DLs by sending samples for analysis together with full instructions for reporting. It collects the reports (OPCW-restricted) and prepares itself the final report. Having all this in mind and knowing that a similar set of samples is sent to one or two other DLs, or as in case of a PT, to more than two other laboratories, the DL is in a position to perform the testing with full professional expertise and confidence.

2 COLLECTION, HANDLING, AND ANALYSIS OF SAMPLES

The CWC describes the general responsibilities for sampling, sample transport to the off-site DLs, confidentiality of samples, sample analysis, and compilation of the report of analysis results of laboratories ⁽¹⁾. The Verification Annex of the CWC, Part II, General Rules of Verification, paragraph E, Conduct of Inspections, and subparagraph Collection, handling and analysis of samples, and paragraphs 52–58 read as follows:

- (52) Representatives of the inspected State Party or of the inspected facility shall take samples

at the request of the inspection team in the presence of inspectors. If so agreed in advance with the representatives of the inspected State Party or of the inspected facility, the inspection team may take samples itself.

- (53) Where possible, the analysis of samples shall be performed on-site. The inspection team shall have the right to perform on-site analysis of samples using approved equipment brought by it. At the request of the inspection team, the inspected State Party shall, in accordance with agreed procedures, provide assistance for the analysis of samples on-site. Alternatively, the inspection team may request that appropriate analysis on-site be performed in its presence.
- (54) The inspected State Party has the right to retain portions of all samples taken or take duplicate samples and be present when samples are analysed on-site.
- (55) The inspection team shall, if it deems it necessary, transfer samples for analysis off-site at laboratories designated by the Organization.
- (56) The Director-General shall have the primary responsibility for the security, integrity and preservation of samples and for ensuring that the confidentiality of samples transferred for analysis off-site is protected. The Director-General shall do so in accordance with procedures, to be considered and approved by the Conference pursuant to Article VIII, paragraph 21 (i), for inclusion in the inspection manual. He shall:

- (a) Establish a stringent regime governing the collection, handling, transport and analysis of samples;
- (b) Certify the laboratories designated to perform different types of analysis;
- (c) Oversee the standardization of equipment and procedures at these designated laboratories, mobile analytical equipment and procedures, and monitor quality control and overall standards in relation to the certification of these laboratories, mobile equipment and procedures; and

- (d) select from among the designated laboratories those which shall perform analytical or other functions in relation to specific investigations.
- (57) When off-site analysis is to be performed, samples shall be analyzed in at least two designated laboratories. The Technical Secretariat shall ensure the expeditious processing of the analysis. The samples shall be accounted for by the Technical Secretariat and any unused samples or portions thereof shall be returned to the Technical Secretariat.
- (58) The Technical Secretariat shall compile the results of the laboratory analysis of samples relevant to compliance with this Convention and include them in the final inspection report. The Technical Secretariat shall include in the report detailed information concerning the equipment and methodology employed by the designated laboratories.

3 QUALITY SYSTEM, ACCREDITATION, AND DESIGNATION

3.1 Standard ISO/IEC 17025 – Basis for Accreditation

The international standard 'General requirements for the competence of testing and calibration laboratories' [ISO/IEC 17025 (1999)] contains all of the requirements that testing and calibration laboratories have to meet if they wish to demonstrate that they operate a quality system, are technically competent, and are able to generate technically valid results. This standard specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, nonstandard methods, and laboratory-developed methods. The standard is applicable to all organizations performing tests and/or calibrations.

In general, the standard is divided into two parts: management requirements and technical requirements. The management requirements define the

following: organization; quality system; document control; review of requests, tenders, and contracts; subcontracting of tests and calibrations; purchasing services and supplies; service to the client; complaints; control of nonconforming testing and/or calibration work; corrective action; preventive action; control of records; internal audits; and management reviews. The technical requirements define personnel; accommodation and environmental conditions; test and calibration methods and method validation; equipment; measurement traceability; sampling; handling of test and calibration results; assuring the quality of test and calibration results; and reporting of results.

The management requirements are stated as follows: The laboratory or the organization of which it is part shall be an entity that can be held legally responsible. It is the responsibility of the laboratory to carry out its testing and calibration activities in such a way as to meet the requirements of this international standard and to satisfy the needs of the client, the regulatory authorities or organizations providing recognition. Further, according to the management requirements the laboratory shall have managerial and technical personnel with the authority and resources needed to carry out their duties and to identify the occurrence of departures from the quality system or from the procedures for performing tests and/or calibrations, and to initiate actions to prevent or minimize such departures; have arrangements to ensure that its management and personnel are free from any undue internal and external commercial, financial and other pressures and influences that may adversely affect the quality of their work; have policies and procedures to ensure the protection of its clients' confidential information and proprietary rights, including procedures for protecting the electronic storage and transmission of results; and have policies and procedures to avoid involvement in any activities that would diminish confidence in its competence, impartiality, judgment or operational integrity.

3.2 Accredited Testing Laboratory

Operating accredited quality system is beneficial to the laboratory in the following ways:

- Accreditation assures clients about the competence of the laboratory in testing at the field defined in the scope
- Laboratory produces controlled and reliable results; confidentiality building
- Documented testing procedures make the work in the laboratory controlled: chemicals, reagents, samples, subsamples, analyses, and analysis results are traceable (chain-of-custody)
- The Quality System is under continuous development because of regular internal audits, annual audits made by the accreditation body, and other improvement actions
- Possible problems occurred that might have affected analysis results must be documented and solved without delay so that similar would not happen any more; this leads to development of the quality system and the methods
- Analytical methods are developed through research and participation in international interlaboratory comparisons; the latter is one way to prove laboratory's competence
- Continuous training of the personnel and motivated personnel.

The laboratory used as an example in this article, VERIFIN, obtained its accreditation as follows. Finnish Accreditation Service (FINAS; www.finas.fi) accredited VERIFIN (testing laboratory number is T073) first in 1996 and again in 2000, using the standard EN45001 and document ISO Guide 25. The scope of accreditation is 'Chemical Testing in the Verification of Chemical Weapons'. In 2000, the quality system was developed to fulfill the requirements of the standard ISO/IEC 17025. FINAS renewed the accreditation in 2001 and 2004 according to it. The accreditation period is four years.

3.3 Designated Laboratory

The Director-General of the OPCW designates an accredited laboratory after it has successfully performed in the PTs. In order to maintain the designation, the DL must annually participate in one PT, and be successful. Two PTs are organized every year (*see Chapter 6*).

The laboratory used as an example in this article, VERIFIN (Finland), obtained its designation in

November 1998. At the same time, the DG designated laboratories from six other countries, one from each: China, the Netherlands, South Korea, Sweden, Switzerland, and the United States. In June 1999, five other laboratories were designated, one from each country: Czech Republic, France, Germany, Poland, and the United Kingdom. Later, laboratories have been designated from four countries, one from each: Belgium, Russian Federation, Singapore, South Africa, Spain, and a second laboratory from the United States.

4 INTERNATIONAL INTERLABORATORY COMPARISON (ROUND-ROBIN) AND PROFICIENCY TESTS

The first international interlaboratory comparison (round-robin) test for the verification of chemical disarmament took place in 1989 as a Finnish initiative. This test and three subsequent tests were coordinated by Finnish CW Project ⁽²⁾. The Provisional Technical Secretariat (PTS) of the Preparatory Commission of the OPCW coordinated the first international interlaboratory comparison test in 1994 ⁽³⁾ and two trial proficiency tests in 1995. The series of official OPCW PTs begun in 1996. Until end of 2004, 16 official PTs have been arranged (*see Chapter 6*).

The OPCW has created the following quality system documents to control the proficiency testing. The documents are 'Standard Operating Procedure for the Organization of OPCW Proficiency Tests' (QDOC/LAB/SOP/PT1), 'Work Instruction for the Preparation of Test Samples for OPCW Proficiency Tests' (QDOC/LAB/WI/PT2), and 'Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests' (QDOC/LAB/WI/PT3).

5 RECOMMENDED OPERATING AND OTHER PROCEDURES

The Recommended Operating Procedures (ROPs) for Sampling and Analysis in the Verification of Chemical Disarmament were proposed by Finland

and were subsequently developed further with international cooperation. After more than 10 years of methods development for the identification of CW agents and their precursors and degradation products ^(4,5), the Finnish Research Project on the Verification of Chemical Disarmament in 1988 and 1989 published Standard Operating Procedures for the Verification of Chemical Disarmament ⁽⁶⁾. In 1989, the first international interlaboratory comparison (round-robin) test for the verification of chemical disarmament was carried out ⁽²⁾. (Renaming of the SOPs to ROPs was proposed in the experts meeting after the round-robin test in Helsinki.) The first round-robin test and the three subsequent tests ⁽²⁾ were aimed at testing and developing the existing procedures, the ROPs. Through sustained international collaboration, the ROPs were upgraded annually ^(2,7). In all these tests and in later proficiency tests, the ROPs have been widely and successfully applied.

From the viewpoint of a DL, sampling will be the responsibility of the OPCW inspectors or SP representatives. Sample preparation and subsequent analysis of the samples is carried out on-site or in a DL in a case where further analysis is required. For a DL, the ROPs ⁽⁷⁾ give instructions for sample preparation and instrumental analysis. Rather than being the best choice for a single analyte or matrix type, ROPs provide an optimal approach for all in an unknown situation. The following ROPs exist for the analysis techniques:

- ROP for the analysis of treaty-related compounds by gas chromatography;
- ROP for the identification of degradation products of treaty-related compounds by microliquid chromatography with flame photometric detection;
- ROP for the identification of methylphosphonic acid and its alkyl derivatives by ion chromatography with conductivity detection;
- ROP for capillary zone electrophoretic analysis of the alkylphosphonic acids and related monoesters using indirect UV detection;
- ROP for identification of treaty-related compounds by gas chromatography/mass spectrometry (GC/MS);
- ROP for the identification of treaty-related compounds and their degradation products by

liquid chromatography/thermospray/mass spectrometry (LC/TS/MS);

- ROP for the identification of treaty-related compounds and their degradation products by liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry (LC/APCI/MS);
- ROP for the identification of treaty-related compounds by Nuclear Magnetic Resonance (NMR) spectrometry;
- ROP for the identification of treaty-related chemicals by gas chromatography/Fourier transform infrared spectrometry.

Laboratories worldwide have different facilities, instrumentation, personnel, and perhaps differing procedures, which means that each laboratory actually requires its own work instructions. These work instructions have to be included in the quality system documentation of the laboratory. In constructing work instructions, the ROPs can offer consultancy ⁽⁷⁾. In addition, there are other useful literature available discussing the methods for analysis of CWC-related chemicals and giving examples on how the laboratories have performed in round-robin tests or PTs ^(8–14).

6 ANALYSIS STRATEGY IN AN OFF-SITE LABORATORY

In verification of presence or absence of chemicals related to the CWC, the analysis of a specific sample in an off-site designated laboratory includes preparation of samples and their analysis by different chromatographic and spectrometric techniques. The analysis strategy employed at VERIFIN is described in the following (Figure 1). Laboratories worldwide may have different instrumentation and perhaps differing sample preparation procedures, which means that each laboratory actually requires its own analysis strategy.

In general, the chromatographic techniques applying specific detectors and selected spectrometric techniques are used in screening to decrease the number of chemical candidates. The screened chemicals may be relevant and are then analyzed using spectrometric techniques in a complementary manner to obtain their unambiguous identification.

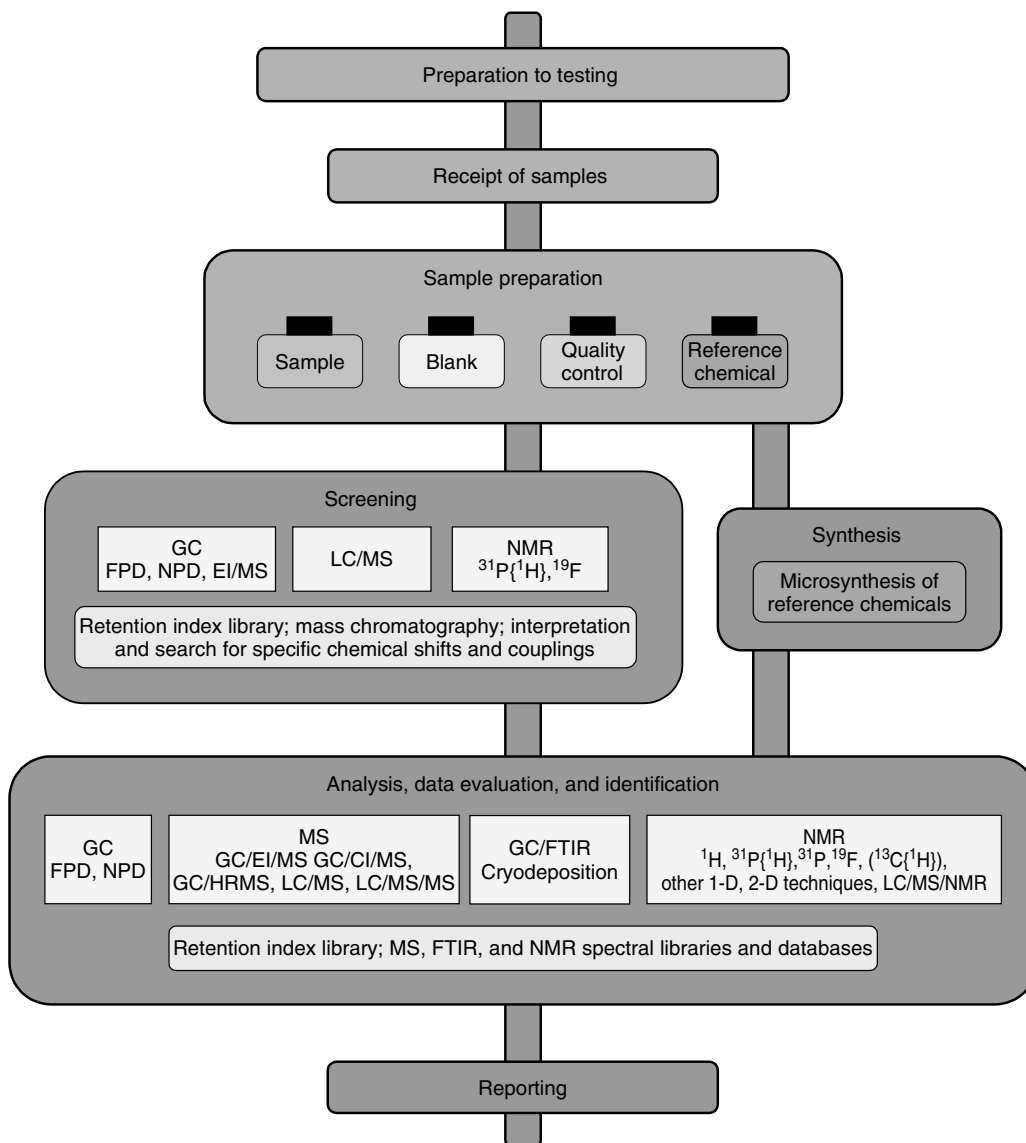


Figure 1. Analysis strategy employed at VERIFIN in testing of CWC-related chemicals

In addition, analysis using the spectrometric techniques may reveal other chemicals relevant to the test. Unambiguous identification of a chemical is obtained if at least two analytical, preferably spectrometric, techniques give consistent results. Analyses are mostly qualitative (identification). Work is conducted according to the ROPs and other documented procedures of demonstrated performance. In the case of a PT, the testing report

is prepared according to the OPCW instructions and sent to the OPCW within 15 days.

6.1 Target Chemicals and Method Range

The toxic chemicals and their precursors that are subject to analysis and identification are listed in Schedules 1–3 (see **Chapter 2**) contained in the

Annex on Chemicals of the CWC ⁽¹⁾. Schedule 1 includes chemicals developed, produced, stockpiled, or used as a chemical weapon as defined above (see **Chapter 1**), and chemicals structurally close to them. Schedule 2 lists three toxic chemicals not included in Schedule 1 and degradation products and precursors of these toxic chemicals as well as of those of Schedule 1. Schedule 3 lists four toxic chemicals and precursors not listed in the other Schedules. Altogether, tens of thousands of chemicals are included in the Schedules.

The Schedules contain mainly organic chemicals with different chemical and physical properties, being neutral chemicals, acids, bases, volatiles, and nonvolatiles, where phosphorus, fluorine, sulfur, chlorine, nitrogen, and oxygen occur frequently.

The procedures referred to in this article are not applicable for analysis of ricin. Analysis of some of the scheduled inorganic chemicals may also require special approach. These are (by the Schedule number) the chemicals 2.B.7, 3.B.5, 3.B.6, 3.B.7, 3.B.12, 3.B.13, and 3.B.14. Riot control agents are not included in the Schedules, but are analyzable with the methods presented.

6.2 Preparation to Testing

The laboratory (VERIFIN) has prepared itself to start analysis of the samples within a short time of the request. It is expected that a reasonable time elapse from the client's first contact until the samples arrive. During this time, the laboratory will make the necessary preparations in order to be ready to receive the samples and start the analyses. In order to be able to start the analyses within a few days from the notice, the laboratory has to maintain its continuous readiness. Amongst others, it means the following.

The instruments and laboratory equipment needed in testing are continuously maintained in good working condition. Also, when the instruments are used in daily research and in methods development, their good condition is a prerequisite. Despite daily use of the instruments, their performance is checked and calibrations are done regularly, and always (during one week) before the testing period. Different instruments need their own performance check (consult e.g. the manufacturers methods). The results of performance checks and calibrations are

registered in logbooks and log sheets. If repair of an instrument is needed, the service is called without delays.

The type and the number of glassware needed in testing are known. New and unused glassware for testing are kept in a stock. The stock is checked and replenished after the testing. This glassware may not be used for other purposes.

The types, qualities, brands, and quantities of solvents, reagents, and other chemicals needed in testing are known, and are kept in stock. After each test, the stock is replenished. Because delivery of chemicals from the supplier may take days or even weeks, this needs to take place well in advance.

6.3 Receipt of Samples

The samples arrive usually by a courier. The receipt of sample package is confirmed (date and sign), which begins the PT period of 15 days. Safety of the workers is taken care by proper protection (fume cupboard, gloves) and a risk assessment is done partly using the information available about the samples. Possible radioactivity can be measured and bacteria killed, if necessary.

The sample-carrying package itself is checked for possible damages from transportation and so are the individual sample containers and bottles. Opening of the package is digital photographed. In case of problems, for example, leakage noticed, the client (OPCW) is contacted.

The samples obtained from the OPCW are coded, which means that the sample origin is not known to the laboratory. At VERIFIN the samples are recoded, and only after that subjected to sample preparation procedures.

6.4 Sample Preparation

The samples and prepared subsamples are coded individually and uniquely according to the documented procedures. The subsamples are linked to each other and marked in a register in such a way that the parent of each subsample is known.

The samples and blanks (if available) are divided into portions allowing multiple sample preparation procedures to meet the requirements of different analytical techniques and chemicals. Quality control

(QC) samples may be prepared. By way of example, a blind sample containing only the solvents/reagents used in preparation of each type of sample is always prepared.

One set of samples is prepared for the analysis techniques relying on GC separation. Samples from water and aqueous extracts can be prepared in straightforward way for analysis by liquid chromatography/mass spectrometry (LC/MS) and NMR. Samples for NMR are normally prepared so as to obtain 5–10-fold concentration. Aqueous liquids are particularly suitable for NMR.

6.5 Screening

The samples are screened for the analytes by GC, GC/MS, LC/MS, and NMR. Results from screening by GC are useful in analyses carried out by the techniques relying on it. The screening is done with element (nitrogen, phosphorus, sulfur)-specific detectors (nitrogen–phosphorus detector, NPD, and flame photometric detector, FPD), together with determination of retention indices (times). This is particularly advantageous, because almost all of the scheduled chemicals are then detectable without interference from hydrocarbons; the other chemicals not containing phosphorus, nitrogen, and sulfur are screened using GC/EI/MS (gas chromatography/electron impact/mass spectrometry) by monitoring ions specific to them. GC/EI/MS is also useful for screening of the family members of nerve agents (Schedule 1.A.1 – 1.A.3) and their derivatized degradation products.

Analogously, LC/MS is useful for screening of the degradation products, and here, no derivatization is required. Screening by $^{31}\text{P}\{^1\text{H}\}$ and/or ^{19}F NMR spectroscopy can reveal phosphorus- and fluorine-containing chemicals. One advantage of LC/MS and NMR over GC-relying techniques is the ability to separate (in LC/MS) and detect polar, non-volatile analytes without sample pretreatment and derivatization.

6.6 Analysis, Data Evaluation, and Identification

Analysis for the identification of known, volatile chemicals, which are fully resolved from the matrix,

is carried out with gas chromatography/low resolution mass spectrometry: GC/EI/MS provides spectrum characteristic for chemical, while GC/CI/MS (chemical ionization) gives the molecular weight information. Problems caused because of strong matrix interference are solved using GC/HRMS (high resolution mass spectrometry) in selective ion monitoring (SIM) mode. Polar and nonvolatile chemicals in aqueous solutions or extracts can be analyzed with LC/MS and LC/MS/MS (tandem mass spectrometry) by various ionization techniques (atmospheric pressure chemical ionization, APCI; electrospray ionization, ESI). Gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR) using cryodeposition offers pure and characteristic (condensed phase) spectra useful for identification. ^1H , $^{31}\text{P}\{^1\text{H}\}$, ^{31}P and ^{19}F and NMR spectra are, as well, characteristic for a chemical, but in ^1H NMR, sample background may cause problems of resonance overlapping. ($^{13}\text{C}\{^1\text{H}\}$ NMR spectroscopy is too insensitive to be used for PT samples ($10\text{ }\mu\text{g/ml}$), but may be useful from higher concentrations, e.g. industrial samples). Use of LC/MS/NMR may, in case of difficult matrix, provide means for identification of a chemical by providing mass spectral and NMR data from the same chemical (peak).

In the case of MS, FTIR, and NMR, the spectrum recorded from a sample is compared with library spectra, and upon fulfillment of certain criteria, identification is obtained. Unambiguous identification of a chemical is obtained if at least two analytical, preferably spectrometric, techniques give consistent results. By way of example, identical identifications obtained by GC/EI/MS and GC/CI/MS are considered to constitute an unambiguous result.

If preliminary results indicate the presence of a Scheduled chemical, which is not in the spectral libraries, its structure elucidation is carried out. The GC/EI/MS, GC/CI/MS, and LC/MS spectra are interpreted. Molecular weight and presence of heteroatoms like sulfur, chlorine, and phosphorus is determined. GC/HRMS and LC/MS/MS assist in interpretation of the fragmentation of ions of interest. Elemental composition of the molecular and fragment ions can be determined with GC/HRMS. Interpretation of GC/FTIR spectrum provides information on functional groups (when reporting in a PT, note that IR spectrum interpretation is not considered sufficient for identification), and the different

types of 1-D and 2-D NMR spectra information on type and number of nuclei, and their location in the molecule, that is, the molecular structure. A consistent result from all used spectrometric techniques is needed for unambiguous identification. The final confirmation is obtained when the suspected chemical is synthesized as a reference chemical, and the spectra recorded from the sample and the synthesized chemicals are identical.

If no CWC-related chemicals are identified from the sample, a so-called on-the-job validation is done. Sample or blank (if available) is spiked with small quantities ($10\text{ }\mu\text{g/g}$; $10\text{ }\mu\text{g/l}$) of CWC-related chemicals. Identical analytical procedure is carried out with the spiked sample. Estimation is then made of the lowest concentration where CWC-related chemicals from the sample could have been analyzed. This information is reported. The default spiking level of test chemicals in PTs is $10\text{ }\mu\text{g/g}$; 10 g/l .

6.7 Reference Data

Reference data used in the identification of CWC-related chemicals in testing are from the following sources.

- OPCW Central analytical Database (OCAD). Version 7 from April 2004 contains 3078 GC retention indices, 2603 mass spectra, 1391 NMR spectra and 710 IR spectra (the database is free for the SPs).
- GC retention index and mass spectral software/database AMDIS (Automated Mass Spectrometry Deconvolution and Identification System). The AMDIS is distributed with the OCAD for free to SPs.
- In-house spectral libraries (data in instrument specific format or on paper).
- Data recorded from authentic reference chemicals synthesized during testing.
- Commercial databases on analytical instruments.

6.8 Reporting

The coordinating researcher prepares the report. The report is prepared using the forms and instructions obtained from the OPCW. Serious mistakes in the report lead to failure in the test, even though the data

itself were correct. Reporting is started at an early stage, preferably during the first week of the testing period. Cross-check of the report is done by at least one other experienced analyst.

The analysis report must include necessary analytical data (chromatographic and spectrometric) supporting the identifications made, describe the sample preparation and analytical methods in detail (or make reference to ROPs, SOPs, or other procedures), and give information on the identified chemicals (CAS registry number, structural formula, and IUPAC or CWC name).

7 DISCUSSION

Testing is teamwork. An experienced analyst is selected as the test manager. The experts of sample preparation and of different analytical areas must collaborate. Colleagues should know the basics of other analytical techniques and sample preparation, and have at least a basic understanding of the chemistry of CWC-related chemicals. During the testing period, regular meetings are held in order to discuss the findings and plan the next actions.

The team should have a flexible approach to problem solving during testing. Experience helps in making right decisions and in the selection of the most suitable sample preparation and analysis methods. The team members maintain and increase their expertise by research and training. Experience on the chemicals, sample matrices, and methods used help make right decisions.

Different analytical techniques (GC, MS, FTIR, NMR) give different views to the problem. As a minimum requirement the GC(RI) and EI/MS are sufficient for identification, but LC/MS, FTIR, and NMR offer independent spectrometric evidence to support to the identification.

ABBREVIATIONS AND ACRONYMS

^1H

Nucleus: Hydrogen-1 or Proton;
Experiment: Hydrogen-1 or
Proton Observation

$^{13}\text{C}\{^1\text{H}\}$	Experiment: Carbon-13 Observation with Simultaneous ^1H Decoupling	GC(RI) HRMS	Gas Chromatography with Retention Index (Determination) High Resolution Mass Spectrometry
^{19}F	Nucleus: Fluorine-19; Experiment: Fluorine-19 Observation	IR ISO/IEC	Infrared International Organization for Standardization/International Electrotechnical Commission
1-D	One-dimensional		
2-D	Two-dimensional		
^{31}P	Nucleus: Phosphorus-31; Experiment: Phosphorus-31 Observation	IUPAC	International Union of Pure and Applied Chemistry
$^{31}\text{P}\{^1\text{H}\}$	Experiment: Phosphorus-31 Observation with Simultaneous ^1H Decoupling	LC LC/MS	Liquid Chromatography Liquid Chromatography/Mass Spectrometry
AMDIS	Automated Mass Spectrometry Deconvolution and Identification System	LC/APCI/MS	Liquid Chromatography/ Atmospheric Pressure Chemical Ionization/Mass Spectrometry
APCI	Atmospheric Pressure Chemical Ionization	LC/MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
APCI/MS	Atmospheric Pressure Chemical Ionization/Mass Spectrometry	LC/MS/NMR	Liquid Chromatography/Mass Spectrometry/Nuclear Magnetic Resonance Spectroscopy
CAS	Chemical Abstracts Service		
CI	Chemical Ionization	LC/TS/MS	Liquid Chromatography/ Thermospray/Mass Spectrometry
CW	Chemical Warfare		
CWC	Chemical Weapons Convention	MS	Mass Spectrometry
DG	Director General	MS/MS	Tandem Mass Spectrometry
DL	Designated Laboratory	NMR	Nuclear Magnetic Resonance
EI	Electron Impact	NPD	Nitrogen-Phosphorus Detector
EI/MS	Electron Impact/Mass Spectrometry	OCAD	OPCW Central Analytical Database
ESI	Electrospray Ionization	OPCW	Organization for the Prohibition of Chemical Weapons
FINAS	Finnish Accreditation Service		
FPD	Flame Photometric Detector	PT	Proficiency Test
FTIR	Fourier Transform Infrared Spectroscopy	PTS	Provisional Technical Secretariat
GC	Gas Chromatography	QC	Quality Control
GC/CI	Gas Chromatography/Chemical Ionization	RI ROP	Retention Index Recommended Operating Procedure
GC/CI/MS	Gas Chromatography/Chemical Ionization/Mass Spectrometry	SIM	Selective Ion Monitoring
GC/EI/MS	Gas Chromatography/Electron Impact/Mass Spectrometry	SOP SP	Standard Operating Procedure State Party
GC/FTIR	Gas Chromatography/Fourier Transform Infrared Spectroscopy	TS	Thermospray
GC/HRMS	Gas Chromatography/High Resolution Mass Spectrometry	TS/MS UV	Thermospray/Mass Spectrometry Ultra Violet
GC/MS	Gas Chromatography/Mass Spectrometry	VERIFIN	Finnish Institute for Verification of the Chemical Weapons Convention

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CHAPTER 9

Sample Preparation for Analysis of Chemicals Related to the Chemical Weapons Convention in an Off-site Laboratory

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1 INTRODUCTION

The reliable verification of chemicals related to the Chemical Weapons Convention (CWC-related chemicals) depends essentially on the collection of good samples and well-planned, effective, and reasonably simple sample preparations suitable to the method of analysis. The collection of good samples is arguably the most critical part of a successful analysis and this is discussed in a separate article (*see Chapter 3*). For its part, proper preparation of samples requires a thorough understanding of the behavior of the various types of chemicals in different sample matrices, both before and during

the sample preparation, and an awareness of the limitations of the chosen instrumental method of analysis.

Mass spectrometry (MS), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy with their numerous applications are the main instrumental techniques for the detection and identification of CWC-related chemicals. During the last few years, however, less laborious techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) have become attractive for the analysis of water samples and extracts where sample preparation is either not required or is relatively simple.

Sample preparation procedures and analytical techniques for the off-site laboratories of the CWC have been developed and tested in five international interlaboratory comparison (round-robin) tests ^(1–5), in two trial proficiency tests, and in more than 14 official proficiency tests (see **Chapter 6**). The Recommended Operating Procedures (ROPs) for sampling and sample preparation ^(6,7) were written and updated on the basis of the results of the round-robin tests. The ROPs (see Table 1) were designed to be comprehensive enough to allow the analysis of all CWC-related chemicals. Accordingly, some of the procedures contain many sample preparation steps. It is also recommended that the ROPs should be used as first choice in the analysis, with other approaches not excluded.

Bulk samples of CWC-related chemicals are analyzed with minimum sample preparation by Fourier transform infrared spectroscopy (see

Chapter 14). At present, no off-site ROPs are available for bulk samples.

Air samples are usually collected to solid adsorbents such as Tenax, XAD resins, graphitized carbons (e.g. Carbpak), active charcoal, or porous polymers (e.g. Chromosorb). The chemicals are eluted from the adsorbent to a liquid or gas phase by liquid–solid elution or extraction or by thermal desorption. Extraction is the most common method. Thermal desorption can be applied when analysis is by GC (gas chromatography) method, and, recently, the use of automated thermal desorption has been proposed to provide increased sensitivity in GC/MS analysis of a wide range of CWC-related chemicals ⁽⁸⁾.

Air sampling for volatile CWC-related chemicals was intensively developed in a Finnish project during the 1980s ^(9–11). A thorough description of the preparation of air samples was included in a review article published in 1990 ⁽¹²⁾. Although other

Table 1. Recommended operating procedures (ROPs) for sampling and sample preparation in the verification of CWC-related chemicals ⁽⁷⁾

	<i>General techniques</i>
GT 5	Recommended operating procedure for cleaning of glassware for environmental sampling
	<i>General sampling</i>
GS 1	General procedures for sampling
GS 2	Recommended operating procedure for packing of samples containing chemical agents
GS 3	Recommended operating procedure for packing of environmental samples
GS 4	Recommended coding procedure for samples
GS 5	Recommended operating procedure for handling background and control samples
GS 6	Recommended procedure for guaranteeing sample integrity
	<i>Sample collection</i>
SC 1	Recommended operating procedure for sampling and analysis of low-volume Tenax air samples
SC 4	Recommended operating procedure for the collection of low-volume XAD-2 air samples
SC 5	Recommended operating procedure for the collection of soil samples
SC 6	Recommended operating procedure for taking aqueous liquid samples
SC 7	Recommended operating procedure for taking liquid samples
SC 8	Recommended operating procedure for the analysis of solid material samples by thermodesorption
	<i>Sample preparation</i>
SP 3	Recommended operating procedure for the preparation of low-volume XAD-2 air samples
SP 4	Recommended operating procedure for the preparation of soil samples
SP 5	Recommended operating procedure for the preparation of wipe samples
SP 6	Recommended operating procedure for the preparation of active charcoal samples
SP 7	Recommended operating procedure for the preparation of aqueous liquid samples
SP 8	Recommended operating procedure for the preparation of concrete samples
SP 9	Recommended operating procedure for the preparation of paint, rubber, and other polymeric samples
SP 10	Quality assurance and quality control in sample preparation

sample matrices have attracted increasing international interest during the past 10 years, the methods presented in the review continue to be highly relevant. Volatile nonpolar CWC-related chemicals can be analyzed directly after elution or thermal desorption from the adsorbent tube. As an example, a quite recent paper compares headspace techniques using Tenax tubes and thermal desorption with solid phase extraction (SPE) using C₈ disks and ethyl acetate as eluent in the analysis of nitrogen mustards in air ⁽¹³⁾.

The ROPs include sampling, sample preparation, and analysis instructions for low-volume Tenax and XAD-2 air samples. Only the preparation of an XAD-2 low-volume air sample is presented in this article, while the thermal desorption of a Tenax tube is described in the context of gas chromatographic analysis (*see Chapter 10*). Active charcoal is such a strong adsorbent that it requires more effective extraction methods than XAD-2 resin or Tenax tubes. Thus, the recoveries of CWC-related chemicals tend to be lower from active charcoal than from other air sampling materials. Furthermore, active charcoal is not usually used for the collection of organophosphorus chemicals. The sample preparation methods for active charcoal samples have not been validated in international round-robin or proficiency tests.

CWC-related chemicals in aqueous liquid samples (water samples) are usually recovered by extraction with an organic solvent. Modern methods such as SPE and solid phase micro extraction (SPME) have also been presented ^(14–24). Organic extractions and these modern methods mainly recover nonpolar CWC-related chemicals, but leave behind the water-soluble and nonvolatile chemicals. These must also be recovered, however, because the agents tend to decompose (hydrolyze) rapidly under conditions in the environment. In the past few years, techniques such as CE and LC, relying on element specific or mass spectrometric detection, have been intensively developed to provide easy and effective ways of recovering these chemicals from water samples with only minor sample preparation ^(25–44). For GC/MS analysis, the water must be displaced and the analytes derivatized.

Soil samples have proved to be of critical importance in confirming the use of chemical warfare agents. Although the preparation of soil samples received little attention in the open literature during

the last decades of last century ^(12,45–51), the past five years have seen much interest in its development ^(36,52–65). Usually, the soil is extracted with an organic solvent to recover the nonpolar CWC-related chemicals, such as nerve and mustard agents and, in view of the probable degradation (hydrolysis), it is also extracted with water to recover the water-soluble polar CWC-related chemicals.

This article reviews the sample preparation methods for analytical techniques used in off-site laboratories. The procedures described are from the ROPs ⁽⁷⁾ and procedures followed at the Finnish Institute for Verification of the Chemical Weapons Convention, VERIFIN. The usefulness of the methods as demonstrated in international comparison and proficiency tests is noted.

2 PREPARATION OF SAMPLES FOR OFF-SITE ANALYSIS

ROPs have been developed and validated for air, aqueous liquid (water), soil, wipe, active charcoal, concrete, paint, rubber, and other polymeric samples for off-site analysis.

2.1 Air Samples

It is recommended that the control zone and the collection zone of a XAD-2 tube be analyzed separately if the breakthrough volume of the most volatile chemicals of interest in the sampling conditions is not known. The control zone is removed from the tube to a small glass vial and sonicated with 2 ml of ethyl acetate for 3 min. The resin is filtered rapidly and the supernatant is analyzed. The trapped chemicals are desorbed from the collection zone by eluting 2 ml of ethyl acetate to the back end of the zone. (It is important to note that the elution direction should be opposite to the collection direction.) The eluate is collected in a glass vial under gravity.

If the breakthrough volume is known and was taken into account during sample collection, the whole tube can be eluted as described above for the collection zone. If required, the sample solutions can be concentrated with mild nitrogen flow. Care must be taken that the sample solutions are never

concentrated to dryness, because certain CWC-related chemicals are adsorbed firmly onto glass surfaces from residues of organic extracts.

It should be noted that highly volatile chemicals such as hydrogen cyanide (CAS 74-90-8) and phosphene (CAS 75-44-5) as well as polar, water-soluble chemicals such as alkylphosphonic and alkylthiophosphonic acids, thiodiglycol (CAS 111-48-8), and aminoalcohols are not quantitatively trapped by XAD-2 resin.

2.2 Aqueous Liquid Samples

The original sample is divided into four or more portions (1–10 ml) if possible, for different analytical purposes, and transferred to screw-capped glass vials. The pH of the first portion (Figure 1, fraction 1A) is determined with a universal pH paper and, if necessary, the sample is neutralized with ammonium hydroxide or hydrochloric acid. The neutralized sample is liquid–liquid extracted with

two portions of water-immiscible organic solvent using a 1:2 volume ratio of solvent to sample. Usually, dichloromethane is used as an extraction solvent and liquid–liquid extraction times are 2–5 min. The extracts are combined and dried. The dried extract is analyzed as such or, where necessary, after approximately 10-fold concentration with mild nitrogen flow. Concentration to dryness must be avoided since certain CWC-related chemicals in residues of organic extracts are firmly adsorbed to glass surfaces. This dichloromethane sample is analyzed for nonpolar CWC-related chemicals.

The aqueous fraction from the first extraction or another portion of the original sample is made alkaline with ammonium hydroxide, and the same liquid–liquid extraction procedure is repeated (Figure 1, fraction 2A). After derivatization, for example with 100 μ l of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, CAS 25561-30-2) for 1 ml extract, the extract is analyzed for alkaline CWC-related chemicals.

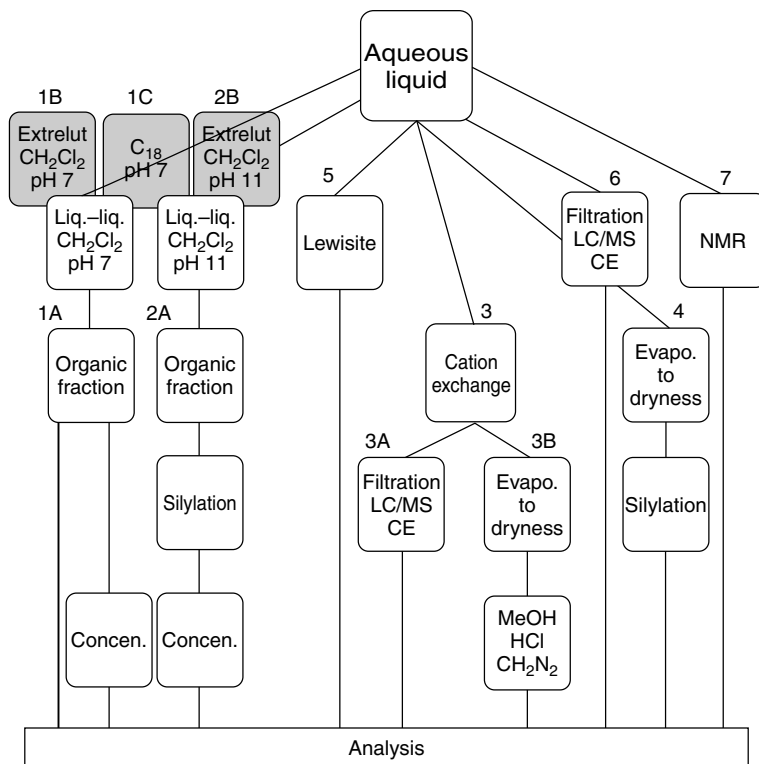


Figure 1. Schematic presentation of the recommended operating procedure for aqueous liquid sample preparation

Alternatively (Figure 1, fractions 1B and 2B), both the neutralized and alkaline liquid–liquid extraction steps can be performed using Extrelut® (Merck) or other corresponding extraction cartridges under similar conditions to those of the liquid–liquid extraction. A portion of the sample is eluted into the cartridge and the analytes of interest are eluted from the sorbent with a suitable solvent (e.g. dichloromethane). The cartridges are used according to the instructions for use delivered by the cartridge manufacturer.

Further, a C₁₈ SPE cartridge may be used (Figure 1, fraction 1C) instead of the neutralized liquid–liquid extraction step. C₁₈ cartridge (100 or 200 mg) is conditioned according to the instructions delivered by the manufacturer and a portion of the sample is eluted through the cartridge. Most of the water deposited in the cartridge is removed by sucking air through and, if necessary, interfering matrix components are removed with a wash solvent (e.g. water). Note that washing may also remove chemicals of interest. The cartridge is eluted with 2 ml of high purity solvent (e.g. acetone). The eluate is dried, analyzed, and, if necessary, concentrated.

The aqueous fraction from the neutral or alkaline liquid–liquid extraction, or another portion of the original sample is eluted slowly through a conditioned SCX (100 or 200 mg) cation-exchange cartridge or a strong cation-exchange resin material (e.g. 1 g AG 50 W-X8 resin, 100–200 mesh, hydrogen form; Figure 1, fraction 3). The pH of the eluate is determined to test the completeness of the cation exchange, which is repeated if necessary. Part of the eluate (0.5–1 ml) is separated and filtered through an HPLC (High Performance Liquid Chromatography) filter for LC/MS and CE analysis (Figure 1, fraction 3A). The rest is evaporated to dryness on a rotary evaporator at 50 °C and 366 mPa. The residue is dissolved in dry acidic methanol and the solution is methylated with diazomethane (Figure 1, fraction 3B). This sample is analyzed for polar alkylphosphonic and alkylthiophosphonic acids. Aminoalcohols remain in the cation exchanger and cannot be recovered.

Another fraction from the neutral or alkaline liquid–liquid extraction, or another portion of the original sample is evaporated to dryness, but without cation exchange, as described above (Figure 1, fraction 4). The residue is dissolved in 1 % triethylamine (TEA, CAS 121-44-8) in methanol and the sample

is sonicated for 5 min. The solvent is transferred to a new vessel and evaporated to dryness with mild nitrogen flow. The new residue is dissolved in acetonitrile or another solvent suitable for silylation. BSTFA is added and the silylation is completed by heating the sample at 60 °C for 30 min. The cooled sample is analyzed for polar CWC-related chemicals such as thiodiglycol and aminoalcohols. Polar alkylphosphonic and alkylthiophosphonic acids can also be recovered from this fraction, but since the procedure does not contain a cation-exchange step, a large amount of inorganic cations in the sample may mean that the recoveries of smaller acids such as methylphosphonic acid (MPA, CAS 993-13-5) are low, or the chemicals may not be recovered at all.

Methyl-*N-tert*-(butyldimethylsilyl)trifluoroacetamide (MTBSTFA, CAS 77377-52-7) is another common silylation reagent used for CWC-related chemicals. BSTFA silylates aminoalcohols such as triethanolamine (CAS 102-71-6) give higher recoveries than MTBSTFA, but the *tert*-butyldimethylsilyl ethers formed when MTBSTFA is used are more stable than the trimethylsilyl ethers formed with BSTFA.

The aqueous fraction from the neutral or alkaline liquid–liquid extraction, or another portion of the original sample is prepared for analysis of the sample for lewisite 1 (CAS 541-25-3), lewisite 2 (CAS 40334-69-8), and lewisite oxide (CAS 3088-37-7) (Figure 1, fraction 5). The pH of the sample is adjusted with hydrochloric acid to pH 2, the sample is filtered, and 0.1 ml of a freshly prepared solution containing 3,4-dimercaptotoluene (DMT, CAS 496-74-2) 5 mg ml⁻¹ in acetone is added. The sample vial is shaken vigorously and allowed to stand for 10 min. Then one milliliter of *n*-hexane is added and the sample is shaken vigorously for 30 s every 10 min for 30 min. The hexane fraction is allowed to separate. The top hexane–water fraction is transferred to a new vial and centrifuged. After centrifugation, the hexane fraction is separated, dried, and submitted for analysis.

In addition, an appropriate portion of the aqueous liquid sample is filtered through an HPLC filter and the filtrate is analyzed by LC/MS and CE (Figure 1, fraction 6), and another portion is prepared for NMR analysis (fraction 7).

2.3 Soil Samples

If possible, the original sample is divided into four or more portions (1–10 g) for different analytical purposes, each placed in a screw-capped glass vial. One portion of the soil (Figure 2, fraction 1) is extracted twice with dichloromethane by sonication, shaking, tumbling, or agitation for 10 min, each time using the same volume in milliliters (ml) of the solvent as the amount of the sample in grams (g). The extracts are centrifuged, filtered, combined, and dried. The dried extract is analyzed as such or, where necessary, after approximately 10-fold concentration with mild nitrogen flow. Concentration to dryness must be avoided since certain CWC-related chemicals in residues of organic extracts are

firmly adsorbed to glass surfaces. This sample is analyzed for nonpolar CWC-related chemicals.

A second portion of soil, or the soil fraction from the dichloromethane extraction (after drying in a fume cupboard for approximately 1 h), is extracted with distilled, deionized water in the manner described above for the dichloromethane extract. Usually, sonication is avoided because it may reduce recoveries of the polar CWC-related chemicals by creating new active sites for analytes in the soil. The extracts are centrifuged, filtered, and combined and the pH is determined.

An appropriate portion of the water extract (Figure 2, fraction 2) is eluted slowly through a conditioned SCX (100 or 200 mg) cation-exchange cartridge or a strong cation-exchange resin material

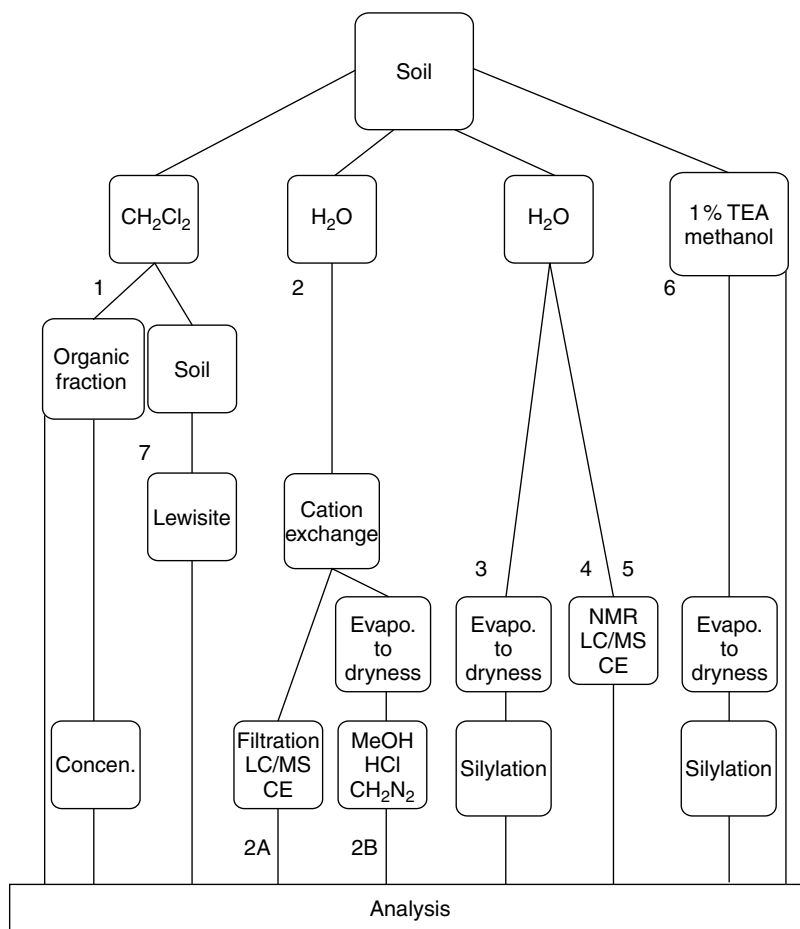


Figure 2. Schematic presentation of the recommended operating procedure for soil sample preparation

(e.g. 1 g AG 50W-X8 resin, 100–200 mesh, hydrogen form). The pH of the eluate is determined to test the completeness of the cation exchange, which is repeated if necessary. Part of the eluate (0.5–1 ml) is separated and filtered through an HPLC filter for LC/MS and CE analysis (Figure 2, fraction 2A). The rest is evaporated to dryness on a rotary evaporator at 50 °C and 366 mPa. The residue is dissolved in dry acidic methanol and the solution is methylated with diazomethane. The methylated solution is analyzed for polar alkylphosphonic and alkylthiophosphonic acids (Figure 2, fraction 2B). Aminoalcohols remain in the cation exchanger and cannot be recovered.

The extraction procedure with water is repeated, or another portion of a water extract is evaporated to dryness, but without cation exchange, as described above. The residue is dissolved in 1 % TEA/methanol and the sample is sonicated for 5 min. The solution is transferred to a new vessel and evaporated to dryness with mild nitrogen flow. The new residue is dissolved in acetonitrile or another solvent suitable for silylation, and silylated with BSTFA. The silylation is completed by heating the sample at 60 °C for 30 min (Figure 2, fraction 3). This fraction is analyzed for polar CWC-related chemicals such as thiodiglycol and aminoalcohols. Polar alkylphosphonic and alkylthiophosphonic acids can also be recovered, but since the procedure does not include a cation-exchange step, if there are large amounts of inorganic cations in the sample, the recoveries of smaller acids such as MPA may be low or they may not be recovered at all.

MTBSTFA is also commonly used as a silylation reagent for the CWC-related chemicals (see Section 2.2).

Next, an appropriate portion of a water extract(s) is filtered through an HPLC filter and analyzed by LC/MS and CE (Figure 2, fraction 4), and another portion is prepared for NMR analysis (fraction 5).

In the third step (Figure 2, fraction 6), a new portion of soil or the soil fraction from the water extraction step is extracted with 1 % TEA/methanol in the manner described above for dichloromethane. A portion of the centrifuged, filtered, and combined TEA/methanol extract is analyzed as such or, where necessary, after approximately 10-fold concentration with mild nitrogen flow. Concentration to dryness must be avoided since certain CWC-related chemicals in residues of organic extracts are firmly

adsorbed to glass surfaces. The rest of the extract is evaporated to dryness on a rotary evaporator at 50 °C and 31 Pa for ca. 10 min and then at 50 °C and 366 mPa for ca. 20 min. The evaporation residue is silylated as described above for the evaporation residue of the water extract. This fraction is analyzed for alkaline CWC-related chemicals such as VX (CAS 50782-69-9).

A fourth sample portion, or the soil portion after dichloromethane extraction, is prepared for analysis of the sample for lewisite 1, lewisite 2, and lewisite oxide (Figure 2, fraction 7). The soil is mixed with hydrochloric acid (e.g. 10 g of soil with ca. 15 ml of HCl). The pH of the water fraction is determined and adjusted to pH 2 if necessary. The pH of the water fraction must be maintained between 1.5 and 2.5 over a period of 30 min. The sample is then shaken vigorously for 30 seconds every five min for 60 min, allowed to settle for an hour, centrifuged, and filtered. To the filtrate is added 0.1 ml of freshly prepared solution containing DMT 5 mg ml⁻¹ in acetone. The sample vial is shaken vigorously and allowed to stand for 10 min, and then 1 ml of *n*-hexane is added and the sample is shaken vigorously for 30 s every 10 min for 30 min. The hexane fraction is allowed to separate. The top hexane–water layer is transferred to a new vial and centrifuged. After centrifugation, the hexane fraction is separated, dried, and submitted for analysis.

2.4 Wipe Samples

The wipe sample is inserted into a glass vial or the original sample container is used as an extraction vessel. If a blank sample is available, a small amount of this is tested for solubility in the selected solvent. A nonpolar organic solvent is poured over the sample. Acetone, ethyl acetate, dichloromethane, and deuterated chloroform (NMR analysis) are all good solvents. Care must be taken that the wipe sample is completely covered with the solvent. The mixture is sonicated for 3 min. The organic phase is quickly decanted and, if necessary, filtered and centrifuged. The extraction procedure is repeated with a second portion of the solvent. The extracts are combined and analyzed (Figure 3, fraction 1) and, if necessary, an appropriate amount of the extract is concentrated approximately 10-fold with mild nitrogen flow. Concentration to dryness must

be avoided since certain CWC-related chemicals in residues of organic extracts are firmly adsorbed to glass surfaces (Figure 3, fraction 1A). This extract is analyzed for nonpolar CWC-related chemicals. Since a wipe sample is not a very adsorptive sample matrix, one can also expect to recover polar CWC-related chemicals, and a 0.5–1 ml portion of the extract can be derivatized with 100 μ l of BSTFA for their analysis (fraction 1B). A suitable portion of the extract can also be prepared for NMR analysis (fraction 1C).

The wipe sample from the organic solvent extraction is then extracted with polar solvent (e.g. water, methanol, or acetonitrile) in the manner described above for nonpolar solvent (Figure 3, fraction 2A). For analysis with GC and GC-hyphenated techniques, the polar extract must be derivatized. A part of the acetonitrile extract can be silylated with BSTFA in the same way as the organic extract of the wipe sample; however, methanol or water extracts

cannot be silylated directly, and must first be evaporated to dryness. An appropriate part of the extract is evaporated to dryness on a rotary evaporator (water extract at 50 °C and 366 mPa for ca. 30 min and methanol extract at 50 °C and 31 Pa for ca. 10 min followed at 50 °C and 366 mPa for ca. 20 min). The methanol extract can also be evaporated with mild nitrogen flow. Then 0.5 ml of acetonitrile and 0.5 ml of BSTFA are added over the evaporation residue and the silylation mixture is heated at 60 °C for 30 min to complete the silylation. This fraction is analyzed for polar CWC-related chemicals such as thiodiglycol, aminoalcohols, and polar alkylphosphonic and alkylthiophosphonic acids.

A suitable portion of a water, methanol, or acetonitrile extract can also be analyzed by LC/MS or CE after filtration through a HPLC filter (Figure 3, fraction 2B), and another portion can be prepared for NMR analysis (fraction 2C).

An appropriate portion of the polar extract of the wipe sample is prepared for analysis of the sample for lewisite 1, lewisite 2, and lewisite oxide (Figure 3, fraction 2D). The pH of the sample is adjusted with hydrochloric acid to pH 2, the sample is filtered, and 0.1 ml of freshly prepared solution containing DMT 5 mg ml⁻¹ in acetone is added. The sample vial is shaken vigorously and allowed to stand for 10 min, 1 ml of *n*-hexane is added, and the sample is shaken vigorously for 30 s every 10 min for 30 min. The hexane fraction is allowed to separate. The top hexane–water layer is transferred to a new vial and centrifuged. After centrifugation, the hexane fraction is separated, dried, and submitted for analysis.

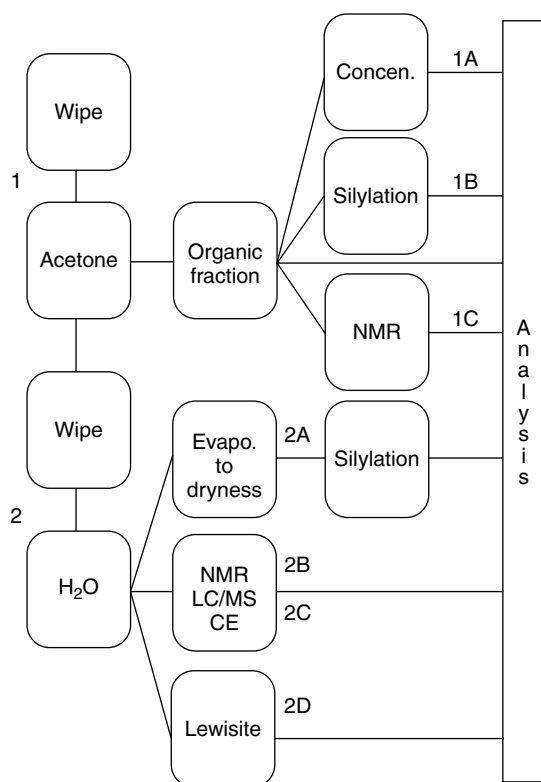


Figure 3. Schematic presentation of the recommended operating procedure for wipe sample preparation

2.5 Active Charcoal Samples

Active charcoal sometimes is doped with various chemicals to modify its properties for a specific purpose. This may cause degradation of the sample chemicals and extraction problems. The matrix may also contain substantial amounts of water. For these reasons, the origin and background of active charcoal samples should always be ascertained.

The active charcoal material is placed in a glass vial. The extraction solvent is added (twice the volume of the charcoal) and the mixture is sonicated for 3 min. Suitable solvents are acetone, dichloromethane, carbon disulfide, and deuterated

chloroform (NMR analysis). The organic phase is quickly decanted and, if necessary, filtered and centrifuged. The extracts are analyzed and, if necessary, an appropriate amount of the extract is concentrated approximately 10-fold with mild nitrogen flow.

The sample or a few grains of it can also be analyzed by thermal desorption (*see Chapter 10*) if the origin and nature of the charcoal material and the sampling procedure are known.

2.6 Concrete Samples

Small pieces of a concrete sample may be extracted as such, but if the sample pieces are large, homogenizing by crushing will be necessary before extraction. The piece of concrete (ca. 10 g) is inserted into a glass bottle, 10 ml of acetone or dichloromethane is added, and the sample mixture is sonicated for 30 min (Figure 4, fraction 1). More solvent must be used if the sample is not covered by the solvent. To keep the bath at ambient temperature, the temperature of the water bath is monitored and water is added, if necessary. The organic phase is quickly decanted and, if necessary, centrifuged and filtered. The extraction procedure is repeated with a second portion of the solvent. The extracts are combined and analyzed and, if necessary, an appropriate amount of the extract is concentrated approximately 10-fold with mild nitrogen flow. Concentration to dryness must be avoided since certain CWC-related chemicals in residues of organic extracts are firmly adsorbed to glass surfaces. This extract is analyzed for nonpolar CWC-related chemicals.

The concrete sample from the organic extraction or a new portion of 10 g of the original sample is then extracted with distilled, deionized water in the manner described above for organic solvent (Figure 4, fraction 2). This sample is analyzed for polar CWC-related chemicals. For analysis with GC and GC-hyphenated techniques, the water extract must first be derivatized: an appropriate part of the extract is evaporated to dryness on a rotary evaporator at 50 °C and 366 mPa for ca. 30 min, and then 0.5 ml of acetonitrile and 0.5 ml of BSTFA are poured over the evaporation residue and the silylation mixture is heated at 60 °C for 30 min to complete the silylation (Figure 4, fraction 2A). This

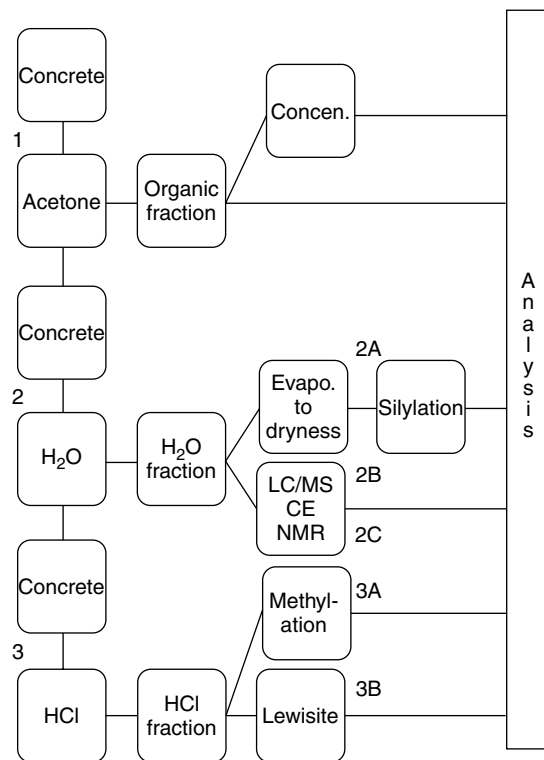


Figure 4. Schematic presentation of the recommended operating procedure for concrete sample preparation

sample is analyzed for polar CWC-related chemicals such as thiodiglycol, aminoalcohols, and polar alkylphosphonic and alkylthiophosphonic acids. The evaporation residue may also be methylated instead: the residue is dissolved in dry acidic methanol and the solution is methylated with diazomethane. This sample is analyzed for polar alkylphosphonic and alkylthiophosphonic acids.

A suitable portion of the water extract can be analyzed by LC/MS or CE after filtration through a HPLC filter (Figure 4, fraction 2B), and another portion can be prepared for NMR analysis (fraction 2C).

The concrete sample from the water extraction or a new portion of 10 g of the original sample is then extracted in a similar way with 1 M HCl solution (Figure 4, fraction 3A). This extract is handled in the same way as the water extract, for the same analytes. Note that silylation of acidic evaporation residues may be difficult, and neutralization of the extract

before evaporation to dryness is recommended if the HCl extract is to be silylated to recover chemicals such as thiodiglycol and aminoalcohols.

An appropriate portion of the HCl extract of the concrete sample is prepared for analysis of the sample for lewisite 1, lewisite 2, and lewisite oxide (Figure 4, fraction 3B). The pH of the sample is adjusted with hydrochloric acid to pH 2, the sample is filtered, and 0.1 ml of a freshly prepared solution containing DMT 5 mg ml^{-1} in acetone is added. The sample vial is shaken vigorously and then allowed to stand for 10 min. After addition of 1 ml of *n*-hexane, the sample is shaken vigorously for 30 s every 10 min for 30 min. The hexane fraction is allowed to separate. The top hexane–water layer is transferred to a new vial and centrifuged. After centrifugation, the hexane fraction is separated, dried, and submitted for analysis.

2.7 Paint, Rubber, and Other Polymeric Samples

The sample is crushed or cut into small pieces, if necessary, and placed into a glass bottle. Then 10 ml of acetone (dichloromethane, acetone- d_6 , or CDCl_3) is added and the sample mixture is sonicated for 10 min (Figure 5, fraction 1). The solvent must be sufficient to cover the sample. Some paint, rubber, and polymeric matrices may, however, be soluble in or may swell in dichloromethane or chloroform, and the solubility of the sample in the extraction solvent should be tested before the solvent is used, especially if a blank sample is available. The organic phase is quickly decanted and, if necessary, centrifuged and filtered. The extraction procedure is repeated with an additional portion of the solvent. The extracts are combined and analyzed and, if necessary, an appropriate amount of the extract is concentrated approximately 10-fold with mild nitrogen flow. Concentration to dryness must be avoided since certain CWC-related chemicals in residues of organic extracts are firmly adsorbed to glass surfaces. This extract is analyzed for nonpolar CWC-related chemicals. Paint, rubber, and other polymeric samples are not very adsorptive sample matrices. For this reason, one could also expect to recover some polar CWC-related chemicals from this fraction. To find these, a portion of 0.5–1 ml of the extract can be derivatized with 100 μl of BSTFA.

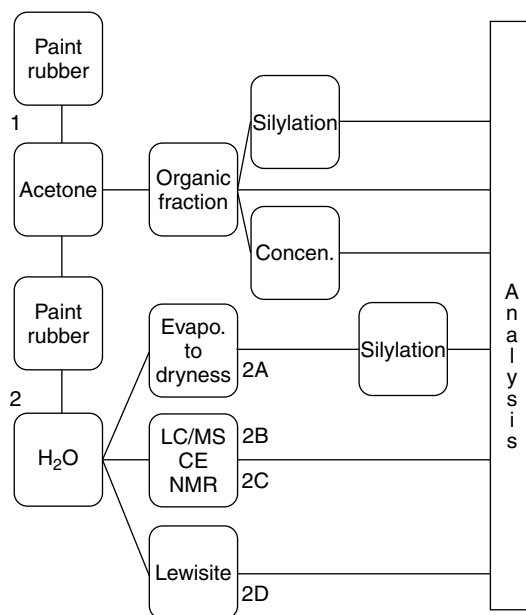


Figure 5. Schematic presentation of the recommended operating procedure for paint, rubber, or other polymeric sample preparation

The paint, rubber, or other polymeric sample from the organic extraction, or a new portion of the original sample is extracted with distilled, deionized water in the manner described above for an organic solvent (Figure 5, fraction 2). This sample is analyzed for polar CWC-related chemicals. For analysis with GC and GC-hyphenated techniques, the polar extract must first be derivatized: an appropriate part of the extract is evaporated to dryness on a rotary evaporator at 50°C and 366 mPa for ca. 30 min. Then 0.5 ml of acetonitrile and 0.5 ml of BSTFA are poured over the evaporation residue and the silylation mixture is heated at 60°C for 30 min to complete the silylation (fraction 2A). This sample is analyzed for polar CWC-related chemicals such as thiodiglycol, aminoalcohols, and polar alkylphosphonic and alkylthiophosphonic acids. The evaporation residue may also be methylated: the residue is dissolved in dry acidic methanol and the solution is methylated with diazomethane. This sample is analyzed for polar alkylphosphonic and alkylthiophosphonic acids.

A suitable portion of the water extract can be analyzed by LC/MS or CE after filtration through

a HPLC filter (Figure 5, fraction 2B) and another portion can be prepared for NMR analysis (fraction 2C).

Another suitable portion of the water extract is prepared for analysis of the sample for lewisite 1, lewisite 2, and lewisite oxide (Figure 5, fraction 2D). The pH of the sample is adjusted with hydrochloric acid to pH 2, the sample is filtered, and 0.1 ml of a freshly prepared solution containing DMT 5 mg ml^{-1} in acetone is added. The sample vial is shaken vigorously and allowed to stand for 10 min. After 1 ml of *n*-hexane is added, the sample is shaken vigorously for 30 s every 10 min for 30 min. The hexane fraction is allowed to separate. The top hexane–water layer is transferred to a new vial and centrifuged. After centrifugation, the hexane fraction is separated, dried, and submitted for analysis.

3 QUALITY CONTROL

Ensuring quality during sample preparation requires that the samples do not come in contact with one another, nor should they be contaminated by chemicals from the laboratory or other samples, and the procedures are shown to be controlled.

Meeting the quality demands of sample preparation requires that the ROPs are followed in all sample preparations.

The purity of solvents and reagents is checked before sample preparation by the same methods used for analysis. The purity of 10-fold concentrated solvents is also tested beforehand. The background generated during the sample preparation procedure is demonstrated preparing a blind sample, by passing the same solvents, same reagents, and similar glassware used in sample preparation through the whole procedure. Similarly, where possible, the background generated by the sample matrix is demonstrated by passing a blank sample through the entire procedure.

It cannot be emphasized too strongly that mistakes in sample preparation may cause failure of the whole analytical procedure, even with the most sophisticated instrumentation. This means that not only must the quality control rules be followed but work must be done unhurriedly and with total concentration.

4 SAFETY

CWC-related chemicals must be handled with great care. Persons handling toxic chemicals must be specially trained for the work. When toxic samples are handled, decontamination solution, protective masks, and autoinjectors of nerve agent antidotes must always be available, and no one must ever work alone. Individual protective gear such as laboratory coats, chemically resistant protective gloves, and safety goggles are essential during sample preparation. Toxic samples must always be prepared in a fume cupboard.

The fume cupboard is cleaned directly after sample preparation. Any samples, organic solvent waste, chlorinated solvent waste, and aqueous wastes that do not require decontamination are collected into separate, clearly marked waste containers. In the same way, paper and consumable wastes that do not require decontamination are collected in a clearly marked waste box. Materials requiring decontamination must be treated with a proper decontamination solution and disposed of in designated waste containers. Glassware and accessories are flushed with decontamination solution and soaked in potassium hydroxide solution and, if not destroyed, they are washed with alkaline nonphosphorus detergent before further cleaning.

5 INTERNATIONAL COMPARISON AND PROFICIENCY TESTS

As described in the introduction of this article, sample preparation procedures for off-site laboratories for the CWC have been developed and tested in international interlaboratory comparison (round-robin) tests ^(1–5), in two trial proficiency tests, and in over ⁽¹⁴⁾ official proficiency tests (see **Chapter 6**). Tables 2 and 3 list the types of samples in these tests. The first three tests were arranged mainly for purposes of method development, the fourth and fifth also for testing and validating of methods. The reports describing these tests (the round-robin books) contain a thorough description of how each of the participating laboratories prepared their samples ^(1–5). Most often, water samples (16 times), organic liquid samples (14 times), or soil samples (12 times) were used as

Table 2. Sample types in the international interlaboratory comparison (round-robin) tests for verification of chemical disarmament arranged for method development and testing

Test	Samples	Comment
Round-robin 1 (1989) ⁽¹⁾	Tenax air samples XAD-2 air samples Soil samples	ROPs were established
Round-robin 2 (1990) ⁽²⁾	Tenax air samples XAD-2 air samples Wipe samples Charcoal samples Aqueous liquid (water) samples	Existing ROPs were tested for air samples ROPs were established
Round-robin 3 (1991) ⁽³⁾	Concrete samples Paint samples Rubber samples	ROPs were established
Round-robin 4 (1993) ⁽⁴⁾	Soil samples Water samples Water samples trapped in a C ₁₈ cartridge	Existing ROPs were validated Quantitative analysis
Round-robin 5 (1994) ⁽⁵⁾	Soil samples Water samples Decontamination solutions	Existing ROPs were tested

Note: The year refers to the dates the tests were carried out

matrices. In the ninth, tenth, and fourteenth official tests, aqueous samples simulating decontamination solutions containing inorganic salts or emulsions were used as sample matrices.

Only in one test (the fourth round-robin) were the identified chemicals quantified ⁽⁴⁾, and then only to facilitate the evaluation of sample preparation methods. The quantitative results varied widely. Weaknesses were revealed in the procedures because all the chemicals were polar and adsorptive. Comparison of the quantitative results as a means of deciding upon the best sample preparation procedures proved to be an unreasonable approach, however, as the total variance in the results was clearly the product of several factors: the sample preparation procedure, the quantitative method, and the instrumental technique. Nevertheless, the exercise proved to have considerable educational value. The test revealed weaknesses in the existing ROPs ⁽⁶⁾ and the methods were subsequently improved to cover the gaps ⁽⁷⁾.

In both the trial and official proficiency tests, the ROPs for sample preparation validated in the round-robin tests proved themselves useful methods to recover CWC-related chemicals spiked at trace level. The participating laboratories had prepared the samples following the ROPs, though sometimes with slight modifications. Many of the laboratories were also able to identify degradation products

or impurities of the spiking chemicals present in only very low concentration. The results show the ROPs to work well in the recovery of chemicals. Where a laboratory had problems, the most common difficulty was the unsuccessful identification of the spiking chemicals or lack of reference data, not improper sample preparation.

An essential requirement in achieving good analytical results is the experience and knowledge of those doing the sample preparation and instrumental analysis. Some examples of incorrect sample preparation revealed in the evaluation of results of the proficiency tests are noted in the following. First, the problems in sample preparation to recover chemicals soluble in organic solvent are presented. Difficulties in recovering water-soluble alcohols and aminoalcohols are then noted, and, finally, mistakes in preparing samples containing water-soluble acids are described. The latter seem to cause the greatest difficulty in sample preparation. The structures of chemicals that caused problems are presented in Figures 6–8.

5.1 Chemicals Soluble in Organic Solvents

In the first trial proficiency test, one laboratory did not find either of the spiking chemicals,

Table 3. Sample types in trial and official proficiency tests arranged by the OPCW for selection and testing of designated laboratories

Test	Samples
Trial Proficiency Test (1995)	Rubber samples Paint samples Soil samples
The Second Trial Proficiency Test (1995)	Organic liquid samples Water samples Soil samples
The First Official Proficiency Test (1996)	Organic liquid samples Water samples Polymer samples
The Second (1996)	Organic liquid samples
The Fifth (1998)	Water samples
The Sixth (1999)	Soil samples
The Seventh (2000)	—
The Eighth (2000)	—
The Twelfth (2002) Official Proficiency Test	—
The Third Official Proficiency Test (1997)	Organic liquid samples Water samples Paint samples
The Fourth Official Proficiency Test (1998)	Water samples Soil samples Wipe samples
The Ninth Official Proficiency Test (2001)	Organic liquid samples Decontamination solutions Emulsion samples
The Tenth Official Proficiency Test (2001)	Organic liquid samples Decontamination solutions
The Eleventh (2002)	Organic liquid samples
The Thirteenth (2003) Official Proficiency Test	Water samples
The Fourteenth Official Proficiency Test (2003)	Organic liquid samples Water samples Decontamination solutions

Note: The year refers to the dates the tests were carried out.

Reports are available from the Organization for the Prohibition of Chemical Weapons (OPCW, www.opcw.org), The Hague, The Netherlands.

sesquimustard (CAS 3563-36-8), and CR (CAS 257-07-8), in a paint sample. The laboratory scratched the paint away from the paint plate surface before extraction, which may have caused the chemicals of interest to escape. Furthermore, the matrix most likely dissolved during the extraction, making the analysis of the extract, with so much interference from the sample matrix, more difficult.

3,3-Dimethyl-2-butanol (DMB, pinacolyl alcohol, CAS 464-07-3) was a spiking chemical in the water sample in the first official proficiency test. One laboratory missed it because of insufficient sample preparation: the water sample was not extracted with dichloromethane, in which the chemical is soluble, but was merely evaporated and the residue

derivatized. DMB was evaporated together with the water and in this way was lost.

In the fifth official proficiency test, one laboratory did not identify the dialkylphosphonate ethyl 1-methyl-2-methoxyethyl methylphosphonate (CAS not available) in the soil sample. The soil reportedly was extracted only with 1 % TEA/methanol. Methods involving successive extractions with dichloromethane are recommended to recover this spiking chemical.

Twenty-five laboratories participated in the sixth official proficiency test where lewisite 1 (CAS 541-25-3) was used as spiking chemical in the organic liquid sample. Five of the seven laboratories that performed sample preparations to derivatize the

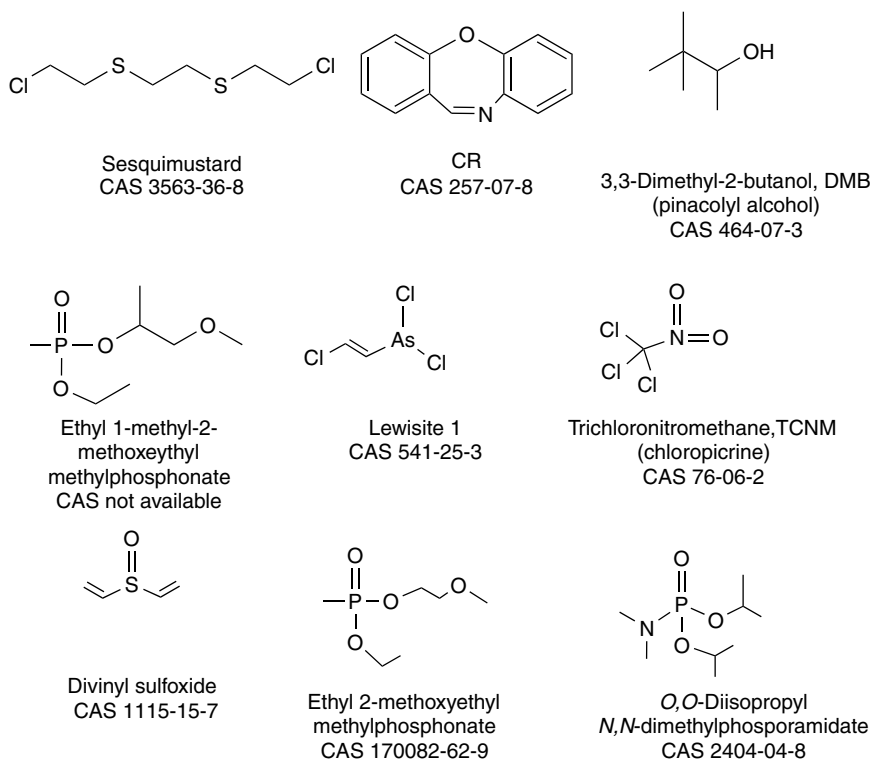


Figure 6. Organic chemicals causing problems in the round-robin and proficiency tests

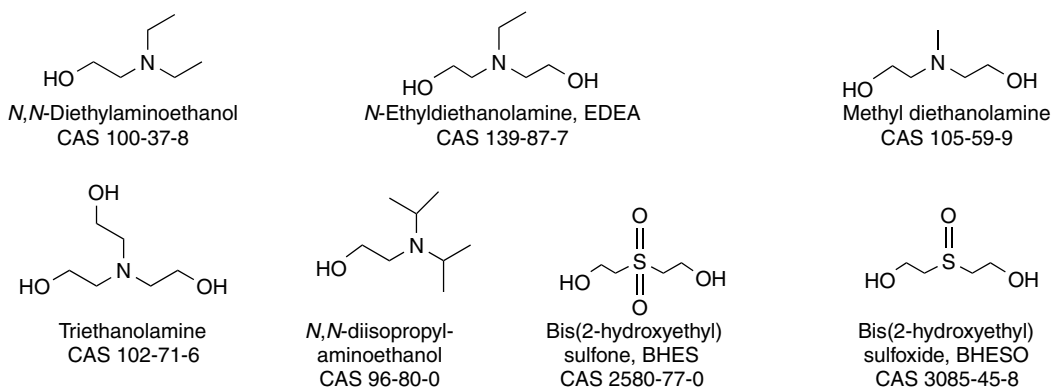


Figure 7. Water-soluble alcohols and aminoalcohols causing problems in the round-robin and proficiency tests

lewisite 1 in the organic liquid were able to identify the chemical. The other laboratories simply did not prepare the sample in a way that would lead to its identification.

The ninth official proficiency test presented a number of problems. Six spiking chemicals were

used but a total of 24 chemicals were found. Seventeen of these 24 were present in one of the three test samples, evidently due to unexpected reactions of the spiking chemicals. One laboratory reported all six spiking chemicals and identified five of them correctly. Three spiking chemicals –

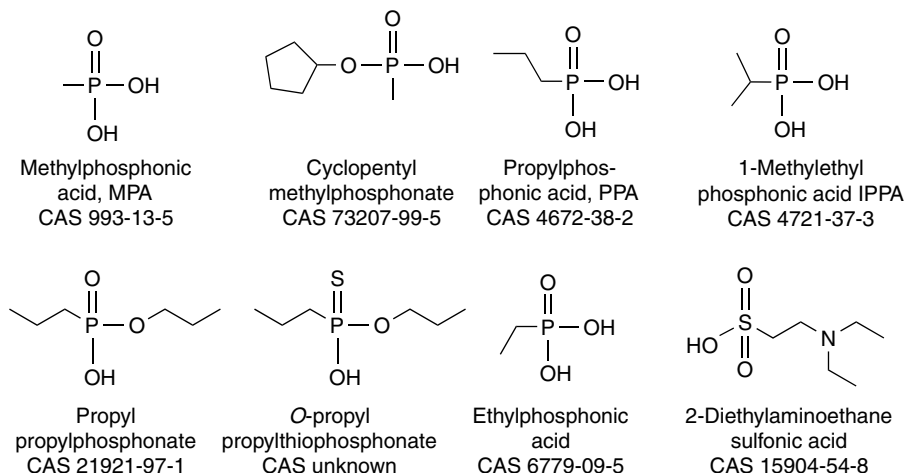


Figure 8. Water-soluble acids causing problems in the round-robin and proficiency tests

bis(2-hydroxyethyl) sulfoxide (BHESO, CAS 3085-45-8), DMB, and trichloronitromethane (TCNM, chloropicrine, CAS 76-06-2) – were not reported by the majority of the 16 participating laboratories.

At least five laboratories may have lost TCNM from the organic liquid sample, probably because the sample was evaporated or concentrated. TCNM and also DMB are relatively volatile and easily lost during these sample preparation stages. Moreover, one laboratory may have removed TCNM from the analytical aliquot during the sample preparation through use of a strong anion-exchange NH_2 SPE cartridge.

In the tenth official proficiency test, five laboratories did not find divinyl sulfoxide (CAS 1115-15-7) in the decontamination solution sample 'D1'. The chemical should have been recovered from the organic extract of the sample. Concentration of the extract, if undertaken, could have helped two laboratories identify this chemical. In one laboratory, false sample preparation was the probable reason for missing it. The laboratory extracted the sample with dichloromethane, evaporated the extract to dryness, dissolved the evaporation residue, and finally silylated it. Usually, organic solvents should not be evaporated to dryness in the recovery of a volatile chemical, and this might be the reason for missing the chemical. Perhaps for this same reason the laboratory missed ethyl 2-methoxyethyl methylphosphonate (CAS 170082-62-9) in the 'D1' sample.

In the twelfth official proficiency test, evaporation was considered to be a reason for one laboratory not finding *O,O*-diisopropyl *N,N*-dimethylphosphoramidate (CAS 2404-04-8) in the organic liquid sample. The laboratory evaporated the sample to dryness and silylated the residue.

5.2 Water-soluble Alcohols and Aminoalcohols

The soil sample was problematic in the second trial proficiency test. Only six of the 15 participating laboratories successfully identified both *N,N*-diethylaminoethanol (CAS 100-37-8) and *N*-ethyl-diethanolamine (EDEA, CAS 139-87-7). For these particular spiking chemicals, efficient TEA/methanol extraction followed by a silylation procedure was essential in the sample preparation. One laboratory missed both spiking chemicals, probably because TEA/methanol extraction was not carried out. Another laboratory also missed them but for a different reason. Instead of TEA, ethyldimethylamine (EDMA, CAS 598-56-1) was used as an extractant, and although this should not have had a dramatic effect on the recoveries of the spiking chemicals, since EDMA should be as good a modifier as TEA, the laboratory then derivatized the 1% EDMA/methanol extract with BSTFA, thus removing any chance of success. Chemicals in alcohol solutions cannot be silylated, and this must

have been the reason why the spiking chemicals were not found.

In the sixth official proficiency test, two laboratories failed to find EDEA and methyl diethanolamine (CAS 105-59-9) in a water sample. One of the laboratories performed only C₁₈ SPE for the water sample and did not carry out an evaporation–derivatization procedure. The other laboratory first performed dichloromethane extraction, and then subjected the residual aqueous fraction to cation exchange, evaporation to dryness, and silylation. These two chemicals may have been retained, at least partially, on the cation-exchange cartridge before evaporation.

In the seventh official proficiency test, one laboratory did not recover triethanolamine (CAS 102-71-6) or *N,N*-diisopropylaminoethanol (CAS 96-80-0). The laboratory performed cation exchange on the water sample, evaporated it to dryness, and methylated it. Both chemicals may have been retained, at least partially, on the cation-exchange cartridge.

In the tenth official proficiency test, the presence of bis(2-hydroxyethyl) sulfone (BHES, CAS 2580-77-0) in the decontamination solution sample 'D2' was missed by one laboratory. The laboratory performed liquid–liquid extraction of the aliquots with dichloromethane and benzene, concentrated the organic extracts, and silylated them. One aliquot of the organic extract was evaporated to dryness, dissolved, methylated, evaporated to dryness, and dissolved again. Evaporation of the water phase to dryness and silylation of the residue was not reported, and the failure to do this might be the reason why the laboratory did not identify BHES using GC-based methods for analysis.

In the twelfth official proficiency test, one laboratory failed to identify BHESO in the organic liquid sample because of unconventional sample preparation. The concentrated extract was silylated with BSTFA at room temperature rather than at the recommended 60 °C.

5.3 Water-soluble Acids

In the second trial proficiency test, one laboratory failed to find MPA and cyclopentyl methylphosphonate (CAS 73207-99-5) in a water sample. The laboratory evidently did not evaporate a portion of the water sample to dryness and derivatize the

evaporation residue, but only derivatized a portion of an alkaline dichloromethane extract. Since the spiking chemicals are polar and acidic, they are not extractable into a nonpolar solvent under alkaline conditions.

In the first official proficiency test, two laboratories did not evaporate and derivatize the evaporation residue of a water sample, and failed to identify propylphosphonic acid (PPA, CAS 4672-38-2). One laboratory employed an SPE method but used a strong cation-exchange cartridge SAX. Weak anion-exchange cartridges are recommended for the SPE method to ensure recovery of the acidic degradation products from water samples. Acids such as PPA are strongly retained on strong anion-exchange cartridges and are hence difficult to elute from the cartridge.

In the third official proficiency test, at least four laboratories failed to find (1-methylethyl)phosphonic acid (IPPA, CAS 4721-37-3) most probably because the water extract was not prepared properly. These laboratories proceeded to neutral and alkaline dichloromethane extraction and some even evaporated the water to dryness. However, recovery of IPPA requires direct analysis of the water sample by LC/MS (or CE) or else cation exchange, evaporation of the sample to dryness, and derivatization (methylation) of the residue.

In the fourth official proficiency test, two laboratories failed to find propyl propylphosphonate (CAS 21921-97-1) and *O*-propyl propylthiophosphonate (CAS registry number unknown) in the water sample. The laboratories followed the ROPs but with slight modifications. One laboratory cation exchanged a portion of the water sample, and after evaporating it to dryness, silylated the residue with 1 % *tert*-butyldimethylchlorosilane (CAS 18162-48-6)/MTBSTFA (10 %) in toluene. The other laboratory extracted a portion of the water sample with dichloromethane, evaporated the water phase to dryness, and dissolved the evaporation residue in diazomethane/ether solution. Another portion of the water sample was extracted with ethyl acetate and evaporated to dryness and the evaporation residue was dissolved in acetone. The acetone solution was then itself evaporated to dryness and silylated with BSTFA/pyridine in acetonitrile. A third portion of the water sample was adjusted to the same pH as the blank water sample and extracted with dichloromethane. After that, the pH was reduced

to ca. 2.5 and the dichloromethane extraction was repeated. This acidic dichloromethane was dried, filtered, and evaporated to dryness before silylation with BSTFA/pyridine in acetonitrile.

It should have been possible to identify the two spiking chemicals in the cation-exchanged and silylated water fraction. However, following cation exchange, higher recoveries are usually obtained for alkylphosphonic and alkylthiophosphonic acids if methylation is used for derivatization. Moreover, the solubility of these chemicals in the silylation mixture is critical for their recovery and therefore either acetonitrile or tetrahydrofuran is typically used as silylation solvent. The solubility of the spiking chemicals in toluene, which the first laboratory chose as a silylation solvent, perhaps was not sufficient. The second laboratory dissolved the first evaporation residue in diazomethane/ether solution, but perhaps the solubility of the spiking chemicals in ether was not sufficient either, especially since the water was not cation exchanged before evaporation. Similarly, before silylation, the second sample fraction was not cation exchanged, and the evaporation residue was dissolved in acetone in which the spiking chemicals are only sparingly soluble. Small amounts of these types of spiking chemicals should be recovered in acidic dichloromethane extracts that are derivatized as such. However, the laboratory first evaporated the dichloromethane extracts to dryness and then silylated the evaporation residue. This might have caused loss of the spiking chemicals.

In the tenth official proficiency test, MPA in the decontamination sample 'D2' was not reported by seven laboratories. In most cases, insufficient sample preparation was the reason for the false negative result. The lack of cation exchange, evaporation to dryness of the water phase, and derivatization of the residue may explain why three laboratories missed the chemical. Three other laboratories evaporated the water phase to dryness and derivatized the residue but did not carry out cation exchange.

In the eleventh official proficiency test, one laboratory missed identifying ethylphosphonic acid (CAS 6779-09-5) in the water sample. Insufficient sample preparation may have been the reason, as the laboratory did not perform cation exchange for the sample before derivatization. Four laboratories successfully identified the chemical without cation

exchange, by simply evaporating the sample aliquot to dryness and silylating the residue. However, the laboratory that failed in the identification used either alkaline or acidic methanol as the first solvent of the evaporation residue. Silylation reagents such as BSTFA easily degrade in moist, acidic, or alkaline conditions, and this may have been another reason why the laboratory missed the chemical.

In the twelfth official proficiency test, 2-diethylaminoethane sulfonic acid (CAS 15904-54-8) caused problems for several laboratories. The chemical was found easily enough in the methylated fraction of the aqueous sample. Silylation was more of a problem because BSTFA was much more unreliable than MTBSTFA in silylation. Six of 19 participating laboratories failed to find the chemical because they performed silylation with BSTFA rather than with methylation.

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ABBREVIATIONS AND ACRONYMS

BHES	Bis(2-hydroxyethyl) sulfone
BHESO	Bis(2-hydroxyethyl) sulfoxide
BSTFA	<i>N,O</i> -bis(Trimethylsilyl)trifluoroacetamide
CE	Capillary Electrophoresis
CR	Dibenz[b,f][1,4]oxazepine
CWC	Chemical Weapons Convention
DMB	3,3-Dimethyl-2-butanol
DMT	3,4-Dimercaptotoluene
EDEA	<i>N</i> -Ethyl-diethanolamine

EDMA	Ethyldimethylamine
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IPPA	(1-methylethyl)phosphonic acid
IR	Infrared
LC	Liquid Chromatography
MPA	Methylphosphonic Acid
MS	Mass Spectrometry
MTBSTFA	Methyl- <i>N</i> -tert(butyldimethylsilyl) trifluoroacetamide
NMR	Nuclear Magnetic Resonance
PPA	Propylphosphonic Acid
ROP	Recommended Operating Procedure
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
TCNM	Trichloronitromethane
TEA	Triethylamine
VERIFIN	Finnish Institute for Verification of the Chemical Weapons Convention

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CHAPTER 10

Gas Chromatography in Screening of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Gas chromatography (GC) is the most widely used analytical technique for the analysis of volatile organic chemicals. The advantages of GC are good resolution, inertness, high sensitivity, and possibilities for selective detection. Although the use of GC with conventional GC detectors has decreased because of the decreased prices of benchtop GC/MS instruments, GC together with selective detectors still has an important role in the analysis of chemicals related to the Chemical Weapons Convention (CWC). Most of these chemicals have a suitable high vapor pressure and thermal stability for GC analysis, or they can be easily derivatized to a volatile form using methylation⁽¹⁾, trimethylsilylation⁽²⁾, *tert*-butyldimethylsilylation⁽³⁾, or pentafluorobenzoylation⁽⁴⁾. Witkiewicz *et al.*⁽⁵⁾ have published

(1996) a comprehensive review concerning various chromatographic applications for the analysis of CWC-related chemicals, whereas Hooijschuur *et al.*⁽⁶⁾ have focused on the literature published between 1996–2002.

GC has been the main separation technique in the international interlaboratory comparison and proficiency tests^(7–14). All participating laboratories used GC, either to determine the retention behavior of analyzed chemicals or as part of the hyphenated techniques. It has been agreed, on the basis of these tests, that the unambiguous identification of CWC-related chemicals has to be based on at least two different analysis techniques, preferably by two different spectrometric analysis techniques, when available, giving consistent results⁽¹⁵⁾. The minimum acceptable data are the correct electron impact mass spectrometry (EIMS) spectrum together

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with the correct retention data. However, GC/EIMS spectra and retention parameters obtained with the same instrument are not regarded as two different techniques ⁽¹⁵⁾.

In verifying the absence or presence of CWC-related chemicals, the analysis of a specified sample includes analyzing subsamples by different chromatographic and spectrometric techniques. In general, the chromatographic techniques together with conventional detectors are useful for screening, and tentative identification and the analyses are in most cases rather qualitative than quantitative. GC methods used for screening purposes should be sensitive and selective and should detect as many CWC-related chemicals as possible in one GC analysis. Sensitivity can be obtained by specific detectors and selectivity by including databases in data handling, containing identification data of relevant chemicals. GC monitoring before GC/MS identification, especially when done with specific detectors, is important in producing useful information from complex mixtures. When a lot of samples must be analyzed, GC can be used to pick out the most valuable samples for a more thorough analysis.

2 INSTRUMENTATION

2.1 Sample Introduction

Injection techniques play an important role in chromatographic separation. Split, splitless, and on-column injection methods have been studied ⁽¹⁶⁾ in order to optimize peak shapes, detection limits, and detector response reproducibility for CWC-related chemicals. A splitless injection at 250 °C with a splitless time of 45–60 s, and using the hot-needle technique with a solvent flush was the most suitable injection method giving satisfactory results. However, an on-column injection has some advantages over other techniques, especially in the analysis of thermolabile chemicals, high boiling chemicals, or chemicals with adsorptive properties. The chemicals will enter the column relatively slowly at a temperature lower than their boiling points. Detection of VX and its homologues, and most dialkyl alkylphosphonates give better results by an on-column injection: the detection limits for VX were about 50 pg with an on-column injection and

2 ng with a splitless injection when the same OV-1 column was used ⁽¹⁷⁾. However, for environmental samples such as soil and plant extracts, an on-column injection may lead to rapid contamination of the analytical column. An exchangeable precolumn coupled with a press-fit connector can be used to protect the analytical column from contamination. In addition, an autosampler cannot always be used with the on-column technique. The split technique provides good separation efficiency for most volatile chemicals such as hydrogen cyanide, phosgene, and cyanogen chloride when the concentrations of these chemicals are high enough. However, discrimination against high boiling components is evident.

The large-volume injection technique together with the on-column injection ^(18,19) and thermal vaporization injection ^(20–22) have been used to improve the sensitivity of GC analysis. Several hundred microliters of the sample can be injected by this technique. Degenhardt–Langelaan *et al.* ⁽²³⁾ determined selected nerve agents at parts per trillion levels in organic extracts of water samples using a large-volume injection. Although this method is very attractive, the optimization of all instrument parameters is quite time-consuming.

The cold trap injection is normally used in the analysis of air samples. During the thermal desorption from Tenax[®] resin ⁽²⁴⁾, the sample chemicals are collected on a cold trap, after which the temperature of cold trap is quickly raised to about 250–300 °C. The chemicals move as a narrow band to the capillary column for a normal gas chromatographic run without an interfering solvent peak. The usefulness of the thermal desorption method for the analysis of CWC-related chemicals has been tested ⁽²⁵⁾. The recoveries at low ng levels were between 81 % and 103 % for sarin, soman, tabun, and mustard gas for volumes of sampled urban air between 5 l and 30 l. VX could not be detected because of its high adsorptive nature. The breakthrough volume of sarin, one of the most volatile nerve agents, was 60 l at the sampling temperature of 23 °C on 100 mg Tenax[®] adsorbent. Tenax[®] tubes loaded with some organophosphates and mustard gas from humidified atmospheres have been stored up to two months without a decrease in recovery ⁽²⁶⁾. Hancock *et al.* ⁽²⁷⁾ developed an automated air sampling analysis system for the monitoring of CWC-related chemicals in air. Air samples were collected on minitubes packed with Tenax[®] TA and then thermally

desorpted into GC without cryofocusing. The use of retention indices (RIs) together with thermal desorption has been reported^(25,28,29). RIs were determined by loading a Tenax® tube with the monitored chemicals and subsequently with a series of index standards.

2.2 Run Conditions

According to the recommended operating procedure for the analysis of CWC-related chemicals by GC⁽²⁴⁾ using SE-54 and OV-1701 columns (25 m × 0.32 or 0.25 mm ID (internal diameter), 0.25 μm film thickness), the following conditions are recommended: Injector temperature 250 °C, detector temperature 280 °C, temperature program: initial temperature 40 °C for 1 min, heating rate 10 °C min⁻¹ to 280 °C for 10 min. However, the GC conditions depend on the injection techniques used, the columns, the sample types, and the analytical demands: if GC is used for rapid sample screening or if maximum resolution is needed, the selected GC conditions are different.

2.3 Columns

The most critical properties of a capillary column are resolution, support inertness, retention reproducibility, thermal stability, and column bleed. To provide fast, reliable, and accurate analysis, it is important that the stationary phase, internal diameter (ID) of the column, film thickness, and length of the column be chosen with a view to the particular application. CWC-related chemicals differ greatly from each other in their chemical and physical properties and thus the selection of the stationary phase is in most cases a compromise between resolution and analysis time. The most suitable stationary phases for the separation of chemicals related to the CWC are listed in Table 1, along with their structures and polarities⁽²⁴⁾.

The nonpolar phases tend to have better characteristics in terms of resistance to oxygen, higher efficiencies, and greater maximum temperatures. Laboratories should have in their use at least two columns of different polarity. SE-54 phase can be regarded a better choice than OV-1 for the nonpolar phase because it provides better peak shapes for fairly polar chemicals such as thiodiglycol and

Table 1. Stationary phases suitable for the analysis of CWC-related chemicals

Polarity	Name	Structure
Low	OV-1	100 % Dimethylpolysiloxane
	SE-54	5 % Phenyl methylpolysiloxane
Intermediate	OV-1701	14 % cyanopropylphenyl methylpolysiloxane
High	CW-20M	Polyethylene glycol

dialkyl alkylphosphonates. The identification cannot always be achieved using retention data obtained on one column. When two columns of different polarities are needed, OV-1701 and SE-54 are a good pair, being sufficiently different in selectivity. An OV-1701 column is recommended for use with a sulfur-selective detector, because mustards and their corresponding primary degradation products elute close to each other on an SE-54 column, and identification may be difficult⁽²⁴⁾. A polar CW-20M column can be used in only limited applications for the analysis of the most polar chemicals. Beck *et al.*⁽³⁰⁾ used a CW-20M column for the analysis of thiodiglycol. Some of the monitored CWC-related chemicals do not elute through this column due to the strong retention and the quite low maximum temperature of the phase material. Huber *et al.*⁽³¹⁾ found that theoretically a maximum of about one million chemicals can be separated by using a combination of three columns with the stationary phases dimethyl polysiloxane, 50 % phenylmethyl polysiloxane, and methylcyanopropyl polysiloxane.

The ID has a direct influence on retention, efficiency, and capacity of the column. The on-column injection technique requires an ID of at least 0.30 mm. A narrow-bore column with an ID of 0.20 mm provides good resolving power with a minimum bleed. It is a good choice for MS analysis as it facilitates a proper adjustment of the carrier gas flow. Narrow-bore columns of limited capacity, however, may be a disadvantage for identification due to a fronting peak shape of overloaded peaks. Columns of an ID between 0.25–0.33 mm can be considered equal for the CWC-related chemicals. Columns of an ID 0.53 mm are useful if the sample contains a limited number of chemicals in widely different concentrations.

Thin film columns (below 0.20 μm) may give poor peak shapes for strongly adsorptive chemicals

such as thiodiglycol, dialkyl alkylphosphonates, and aminoalcohols, especially at a low concentration level. Thick-film columns are ideal for the separation of very volatile chemicals such as hydrogen cyanide, phosgene, and cyanogen chloride. They are unsuitable, however, for higher molecular-weight chemicals such as BZ. Standard-film thickness (0.25–0.33 μm) offers the best compromise between resolution and sample capacity.

The selection of column length depends on the required resolution and analysis time. Short columns (10–15 m) are useful for samples containing a relatively small number of chemicals and also for screening purposes. To keep the analysis time short and to minimize the adsorption, a 50 m column is useful only for very complex separations requiring maximum resolution. Intermediate column lengths of 25–30 m, providing sufficient separation power simultaneously with reasonable analysis time, are in most cases used for the separation of CWC-related chemicals.

2.4 Detectors

The critical properties of detectors are sensitivity, selectivity and linearity of response, reproducibility, and reliability of operation. Spectrometric detectors, MS and Fourier transform infrared (FTIR), are described in more detail in other sections. The following detectors are useful for the detection of CWC-related chemicals.

The flame ionization detector (FID) is currently the most popular universal detector, having both a wide linear range and great reliability. This detector has been widely used for the detection of CWC-related chemicals^(26,32–34). The detection limits for CWC-related chemicals are about 0.1 ng, but the sample composition has a significant influence on the sensitivity: when baseline resolution can be obtained, high sensitivity is the result. In most cases, chromatographic interferences are the main problem with the FID. The sensitivity is poor for some chemicals such as phosgene and hydrogen cyanide. Despite the great separation power of capillary columns, the analysis at trace level in some matrices may be difficult if only the FID is used.

Many selective detectors have been used for monitoring CWC-related chemicals because these chemicals are rich in heteroatoms. In most cases,

they contain from two to four elements such as P, S, O, N, Cl, As, and F. Chromatograms are simplified with these detectors and the sensitivity of analysis is increased.

The photoionization detector (PID) is a selective detector, with the observed response being dependent on the ionization efficiency of the detected chemicals. The PID has a wide linear range of seven orders of magnitude. Any chemical with an ionization potential below 12 eV will give a response. The PID/FID response ratio shows PID to be a more sensitive detector for sulfur-containing CWC-related chemicals such as mustard gas and its homologues for tabun and for chemicals containing double bonds such as lewisites⁽²⁸⁾. Suryanarayana *et al.*⁽³⁵⁾ used PID for the measurement of the time weighted average level of mustard gas in air samples. Because no detector gases are needed, the PID may be useful for some on-site applications.

Phosphorus-selective detectors are of greatest importance in verification analysis. The nitrogen–phosphorus detector (NPD) is very sensitive and selective toward chemicals containing phosphorus and/or nitrogen, reaching detection limits of about 0.5–5 pg and 10–100 pg, respectively. Arsenic-containing chemicals such as lewisites are also detectable with the NPD but the sensitivity is worse than with the FID. The sensitivity of the NPD depends on the temperature of the NPD bead and can change significantly over time, which must be taken into account in the quantitation. In addition, some models of the NPD are not compatible with chlorinated solvents and silylation reagents⁽³⁶⁾. The NPD is normally used in the analysis of nerve agents at trace level^(37–39), but also for the detection of nitrogen mustards⁽⁴⁰⁾.

The flame photometric detector (FPD) is the most widely used detector for sulfur-containing chemicals. Besides sulfur, it is selective for phosphorus, which makes it a very suitable detector for both nerve agents and sulfur-containing mustards^(22,30,41–43). Tomkins *et al.*⁽⁴⁴⁾ detected lewisite oxide as 1,3-propanedithiol derivative with FPD. For sulfur, however, the detector is not linear and suffers from quenching by co-eluting hydrocarbons. Detection limits are about 5–50 pg of phosphorus s^{-1} and about 50–500 pg of sulfur s^{-1} . Two-channel detectors operating simultaneously in the S- and P-mode are also commercially available. The highest operating temperature for many models

is 250 °C. This prevents the detection of higher boiling chemicals and leads to contamination of the detector especially when high concentrations of trimethylsilyl (TMS) derivatizing reagents are used ⁽³⁶⁾.

The detection limits of a pulsed flame photometric detector (PFPD) are much better than those of any conventional FPD, and in addition the detector does not suffer the quenching of co-eluting hydrocarbon chemicals ⁽⁴⁵⁾. The ability to also detect arsenic or nitrogen containing chemicals makes the PFPD very useful for the screening of CWC-chemicals. Frishman and Amiraw ⁽⁴⁶⁾ used fast GC equipped with a short capillary column (1.5 m) and PFPD for the analysis of air samples. A complete analysis cycle time of 30 s was demonstrated. Killelea and Aldstadt ⁽⁴⁷⁾ used PFPD in the arsenic selective mode for the analysis of organoarsenic chemicals.

An alternative to FPD in the sulfur mode is the sulfur chemiluminescence detector (SCD) ⁽⁴⁸⁾. This detector works by forming sulfur monoxide in a reducing flame. Sulfur monoxide is detected by its chemiluminescent reaction with ozone. The SCD is at least one order of magnitude more sensitive than most FPDs. It provides a linear response with high selectivity and does not suffer considerably from quenching.

The electron capture detector (ECD) responds to chemicals capable of reacting with thermal electrons to form negative ions from halogen containing chemicals (the order of increasing response is $F < Cl < Br < I$), and also nitro and α -dicarbonyl groups ⁽⁴⁹⁾. In principle, the ECD is useful for the analysis of some CWC-related chemicals. Unfortunately, environmental samples usually contain a lot of other ECD active material, which seriously interferes with the identification. The very poor sensitivity of the ECD for fluoridate nerve agents suggests the use of NPD or FPD for these chemicals ⁽⁵⁰⁾. Several CWC-related chemicals containing chlorine atoms, for example, vesicants, are detectable with the ECD. Their degradation products are not detected by ECD, so the use of sulfur-selective detectors such as FPD or SCD is recommended for the detection of these chemicals due to their better sensitivity and selectivity.

One of the most attractive detectors for screening of CWC-related chemicals is the atomic emission detector (AED), which is capable of detecting selectively any element below the nanogram level. The

sensitivity and other properties of the AED are compared to other detectors for a variety of analytes in a number of papers ^(36,51,52). With this detector, it is also possible to determine an approximate empirical formula for unknown chemicals ^(53,54). Mazurek *et al.* ⁽⁵⁵⁾ utilized this feature of the detector together with MS-data for the identification of 30 transformation products of mustard gas in a block collected from the Baltic Sea. The AED relies on a microwave-induced helium plasma containing a novel reentrant cavity with a photodiode array-based optical emission spectrometer ⁽⁵⁶⁾. Very-high-purity helium is required as a carrier and plasma gas. Leaks are insidious sources of error, especially when oxygen or nitrogen is being measured. The information obtained from the AED is very useful for unknown chemicals, when the data has to be collected from various techniques for structure elucidation. Nowadays, the AED has been used widely for the detection of CWC-related chemicals ^(57–64).

The usefulness of selective detectors is well demonstrated by the analysis of a spiked sample with the FID and selective detectors. A diesel fuel solution (0.03 %) containing three CWC-related chemicals at low parts per million level is first analyzed with FID (Figure 1). It is clear that the identification of the chemicals of interest is not possible in

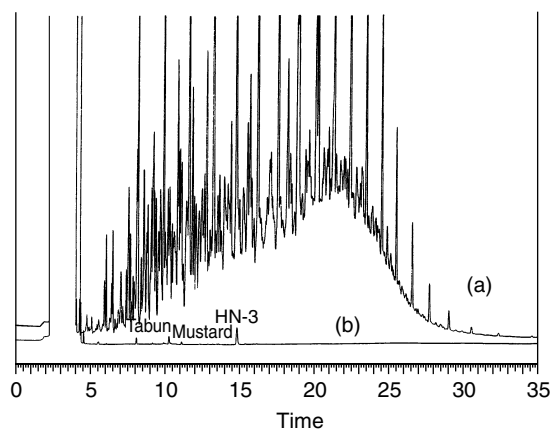


Figure 1. Chromatogram recorded with FID (a) from an ethyl acetate solution containing diesel fuel (0.03 %), tabun (5 ng), mustard (3 ng), and HN-3 (20 ng) and (b) is from a similar solution but with no diesel fuel

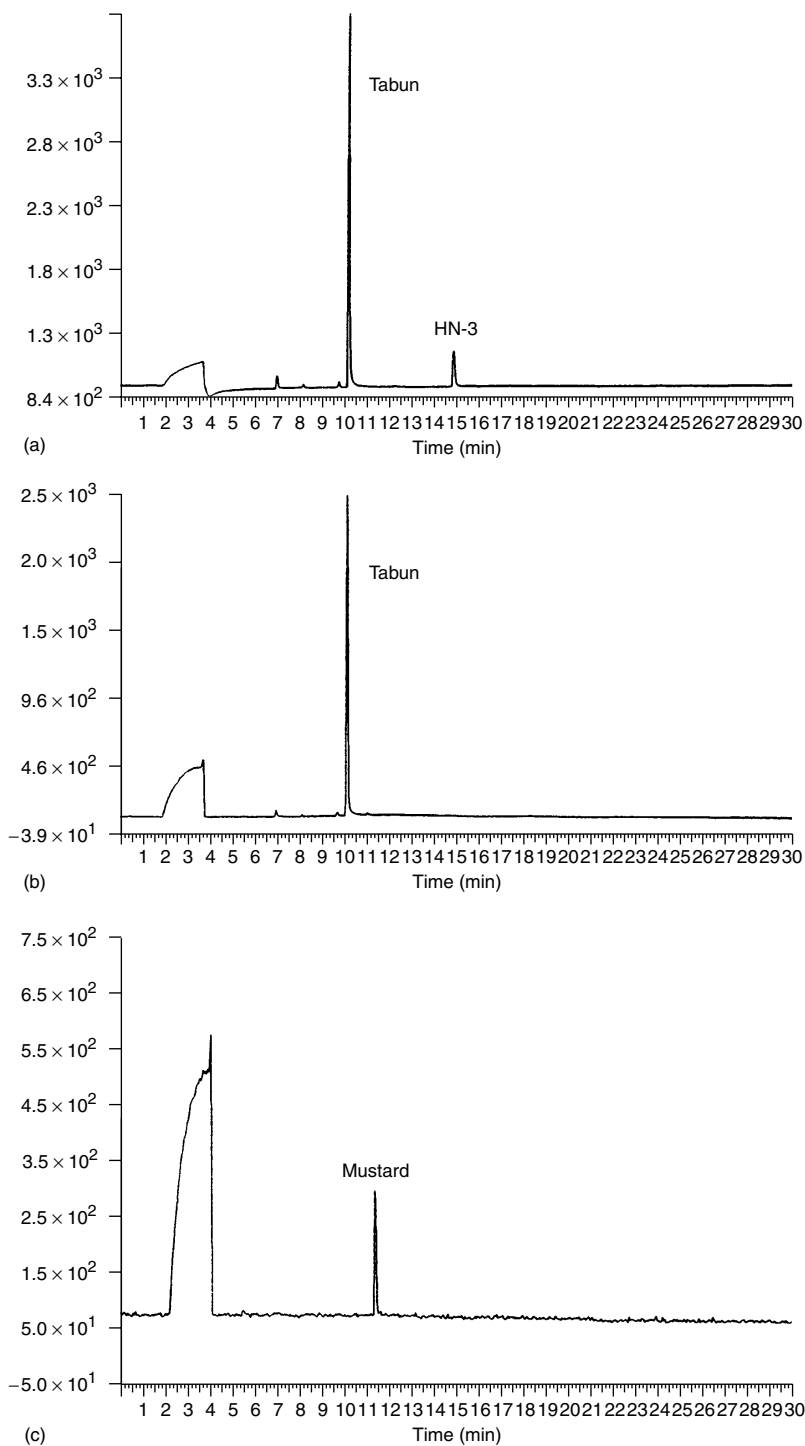


Figure 2. (a) NPD, (b) FPD-P, and (c) FPD-S chromatograms from the same solution as Figure 1(a)

this case. The chromatograms are clearly simplified, when the same sample is analyzed with the two-channel instrument (Figure 2), where the SE-54 column is splitted between NPD and FPD, operating simultaneously in the S- and P-mode.

3 IDENTIFICATION METHODS

3.1 Absolute Retention Times

In most cases, absolute retention times are used for the identification. Modern GCs have high precision and accuracy of retention times within $\pm 0.05\%$, or better can be obtained in subsequent runs during the same day. When the analysis is repeated by another instrument, the deviation may be of several percent. The reproducibility of absolute retention times is strongly dependent on the proper adjustment of all chromatographic parameters. In addition, the column properties are not exactly the same even when similar columns from the same manufacturer are used. On the other hand, absolute retention times are the most useful identification parameters if a few chemicals are to be monitored and the background is low. This method requires frequent calibration because even small changes in chromatographic conditions will influence the absolute retention times.

3.2 Relative Retention Times

For better reproducibility of the retention parameters, relative retention times referenced to a standard chemical have been used in several GC applications. The added reference chemical should be chosen so that it elutes in the middle of the chromatogram. There is always some degree of error in the retention time measurement, and the relative retention times of chemicals having short or long retention times might not be very accurate. To obtain better reproducibility, it is recommended that several different reference chemicals are used for the calculation of relative retention times. However, in this case the use of RIs becomes more attractive. It is a much more reliable to use RIs than absolute or relative retention times because in retention indices (RI) measurements, the retention is measured relative to a homologue series.

3.3 Retention Indices

The reproducibility and reliability of RIs makes it possible to create RI libraries and the identification can be achieved without authentic reference chemicals. The reliability and simplicity of RI monitoring is increased significantly by using a computer program that searches for the RI pattern, calculates the RIs for all peaks in the chromatogram, and then compares the indices with the library data. In addition to the identification of target chemicals, RIs can also be used to locate the interesting peaks between different kinds of GC-based analytical techniques⁽⁶⁵⁾. In this way, it is possible to ensure that all GC-based techniques used for identification focus on the same peaks even in samples with a complex mixture of chemicals.

The RI system proposed by Kovats⁽⁶⁶⁾ was determined at constant temperature using a homologue series of *n*-alkanes (C-series) for calibration. With mixtures that embrace a wide range of boiling points, several isothermal runs are needed. In these cases, the use of linear RIs is more useful, because this method allows determination of indices for rapidly and slowly eluting chemicals in a single analysis. The column temperature is programmed linearly, and the RI of an unknown peak in the chromatogram is calculated from the retention times of the two alkanes eluted on both sides of the chemical⁽⁶⁷⁾. The distance between each pair of standard peaks is taken as equal, and the linear RIs are calculated by a linear polygon method as shown in Equation (1).

$$RI_C = 100C_n + 100(C_{n+i} - C_n) \frac{t_{R(x)} - t_{R(n)}}{t_{R(n+i)} - t_{R(n)}} \quad (1)$$

Where C_n and C_{n+i} are the carbon numbers of the RI standard eluted before and after the unknown chemical, $t_{R(x)}$ is the retention time of the unknown chemical, and $t_{R(n)}$ and $t_{R(n+i)}$ are retention times of C_n and C_{n+i} respectively.

The C-series are the most commonly used RI standards. The RIs of nonpolar substances on nonpolar stationary phases show an almost linear dependence on column temperature. Investigations with other phases and substances suggest, however, that in general the dependence on temperature is not linear but hyperbolic⁽⁶⁸⁾. Alkanes are not detectable with selective detectors that have to be used for

trace analysis. For the specific purpose of identifying of CWC-related chemicals, several series of phosphorus containing retention standards have been synthesized^(69,70). These standards contain an *n*-alkane moiety but also phosphorus, sulfur, and in most cases fluorine, for easy detection with selective detectors. The most stable and suitable moiety for the analysis of CWC-related chemicals is the M-series, alkyl bis(trifluoromethyl)phosphine sulfides, $(\text{CF}_3)_2\text{P}(\text{S})(\text{CH}_2)_n\text{CH}_3$, where $(n = 2, \dots, 19)$ ⁽⁷¹⁾.

3.3.1 Reproducibility of Retention Indices

The reproducibility of the RIs is essential to the reliable screening and identification procedure for CWC-related chemicals. The effects of the following parameters on the reproducibility have been evaluated^(72,73): (1) carrier gas flow rate; (2) starting point of the temperature program; (3) temperature programming rate; (4) injection volume; (5) injection mode; (6) different operator; (7) one- and two-index standard series in the same run; (8) reduced number of index standards; (9) use of external standards; (10) sample solvent; (11) columns from the same and different manufacturers; (12) column length; (13) ID of the column; (14) relative peak area compared with the nearest index standard; (15) repeated use of the same column; (16) concentration of the sample; (17) background; (18) multistep temperature program; (19) model of the instrument; and (20) different laboratories. The C- and M-series were used as index standards and the test chemicals studied were sarin, soman, tabun, VX, mustard gas, CS, CR, and CN. A fused-silica capillary column with an SE-54 stationary phase was used.

The results of the evaluation showed the RIs to vary only slightly with the different operator, the different instrument of the same manufacturer with the same column in one or several laboratories, column individuals of the same manufacturer, solvents (diethyl ether, acetone, and ethyl acetate), and use of a multistep temperature program. There were no significant differences in the reproducibility of RIs determined using different index standard series. M-standards are relatively nonpolar and differ little in their behavior from C-standards on a nonpolar SE-54 phase. When the number of index standard components was reduced, the RI values

also differed. However, the reproducibility remained good. According to this study, the external standard method, where the retention times of the standards are measured in a separate calibration run, may be used, but this requires very good reproducibility of chromatographic conditions and frequent calibration. The most critical parameters affecting the indices were the carrier gas flow rate, the temperature programming rate, and the properties of the column (e.g. length, ID, and film thickness). The influence was greater at the end of the temperature program than at the beginning. Of the model chemicals, VX and the weakly volatile CR were more affected by almost every investigated parameter.

3.3.2 Retention Index Libraries

The application of RIs in the identification of organic chemicals has been extensively investigated in two comprehensive reviews^(74,75). The RIs of some CWC-related chemicals determined against C-series using an on-column injection have been reported^(29,33,76–78). Sokolowski *et al.*^(79–83) and Sliwakowski⁽⁸⁴⁾ reported RIs for selected CWC-related chemicals determined against M-series. A comprehensive RI library was first published by the Finnish Institute for Verification of the CWC^(85,24). RIs have been determined against both M-series and C-series using two different column types (SE-54 and OV-1701: 25 m, 0.32 mm ID and 0.25 μm film thickness), with the following chromatographic conditions: carrier gas helium 1.5 mL min⁻¹, splitless injection with splitless time 45 s, temperature program 40 °C (1 min), and heating rate 10 °C min⁻¹ to 280 °C for 10 min. The index values added to the library were the mean values of three measurements. A standard deviation for RI values was typically less than 0.5 index units.

The correlation between RIs determined using the M-series and the C-series is good, and the C-series indices (RI_C) can be calculated from the M-series (RI_M) indices using the equation⁽⁸⁶⁾ $\text{RI}_\text{C} = 0.9666\text{RI}_\text{M} + 487.6$. Analysis of library values of RIs for the SE-54 column within M_4 – M_{20} and C_8 – C_{24} (100 chemicals) using linear regression, gave correlation 0.99991, and the standard deviation of the error between measured and calculated values was 3.5 index units. A collection of RI values

of CWC-related chemicals from various sources is given in Appendix 1.

For reliable creation of the RI library, all RIs of authentic chemicals determined by the Finnish Institute for Verification of the CWC (see Appendix 1, columns I, II, VI, and VIII) are checked against the RIs of special test chemicals obtained under the same operating conditions. The RIs of library chemicals determined at the beginning and at the end of the library creation must be comparable and consistent. The RI values of test chemicals in Table 2 are the mean values recorded during the creation of the RI library by running the test solution after every sixth run. This practice ensures the quality of the library and the analyst can estimate the correctness of the recorded RI values, by running first the test solution and comparing the RI values of the test chemicals with those presented in Table 2.

Nowadays, the most comprehensive RI library of CWC-related chemicals is available in the OPCW Central Analytical Database (OCAD)⁽⁸⁷⁾, containing nearly 2000 RIs. These values have been determined and provided to the OPCW by the following States Parties: France, Czech Republic, Finland, Netherlands, Switzerland, India, Japan, and USA. The validation group of the OPCW has validated the given data and all accepted RIs have been added in the Database. RIs have been determined against C-series using SE-54-type phase material. In most cases, the indices were measured with a column of 25 or 30 m, 0.25 or 0.32 mm ID, and 0.25 μ m film thickness. The chromatographic conditions were: carrier gas helium 1.0 mL min⁻¹, splitless injection with splitless time 1.0 min, temperature program 40 °C (1 min), and heating rate of 10 °C min⁻¹ to 280 °C for 10 min. In Appendix 2, RIs of OCAD are presented. When more than one RI value is available in the database, the mean value is given.

Because the number of CWC-related chemicals is large, a lot of work is needed before all relevant chemicals are synthesized and the reference data is recorded. Many indices may be predicted from the previously determined index values. The RI of the homologue increases about 100 index units when a straight carbon chain is lengthened by one CH₂ unit. Thus for these kinds of homologues, the RIs of the long carbon chain (six or more carbons) homologues can be predicted quite accurately from the RI of the homologues having three to five carbons. The determined and predicted RI values for three different homologue series differed only by 1.3 index units at maximum⁽⁸⁶⁾. Also, the RIs for homologues containing branched carbon chain ester groups can be predicted relatively well by using RIs of the OCAD as source material.

For most accurate results, the analysis should be carried out under conditions identical to those used for the creating the RI libraries. However, it has been reported that the analytical and column parameters can be changed without altering RIs as long as the initial oven temperature, phase ratio, and the rt_0/β ratio, (r , t_0 , and β are oven heating rate, dead time, and phase ratio respectively) are kept unchanged. The RI values can be reproduced within few index units for two columns of different length and ID, working under different heating rates with the same or different carrier gas at different gas flow rates⁽⁸⁸⁾.

4 QUALITY CONTROL

According to the recommended operating procedure for the analysis of CWC-related chemicals by GC⁽²⁴⁾, the solvent blank, sensitivity, and column performance tests should be carried out to check

Table 2. RIs of test compounds

	M-series		C-series	
	SE-54	OV-1701	SE-54	OV-1701
<i>do</i> -Dimethyl methylphosphonate	400.0	488.8	879.9	1044.4
2,6-Dimethylphenol	644.7	748.4	1111.8	1288.8
5-Chloro-2-methylaniline	850.3	974.5	1308.2	1508.0
Tri- <i>n</i> -butylphosphate	1213.3	1301.5	1659.3	1824.9
Dibenzothiophene	1330.9	1426.3	1772.9	1951.1
Malathion	1551.1	1722.5	1988.3	2241.7
Methyl stearate	1696.5	1689.1	2132.3	2207.5

the performance and sensitivity of the instruments, the accuracy of identification parameters, and cross contamination.

A solvent blank is used to eliminate the possibility of contamination arising from outside the sample, for example, from the syringe or from the instrument. It is recommended to use the sample solvent as solvent blank and run this test at the beginning and at the end of each sample series and whenever contamination is suspected. The memory effect is possible, for example, in the analysis of alkyl phosphonic acids. If the derivatization of these chemicals has not been complete, the nonderivatized acids are adsorbed on the injector liner. A silylation reagent may in such case react *in situ* with the adsorbed acids in the liner yielding false positive results.

A sensitivity test solution is used to check the sensitivity of the detectors. The used test chemicals should not adsorb on the column material at low concentration levels. It is recommended that test solutions supplied from the instrument manufacturer are used. If these are not available, the test chemicals presented in Table 3 are suitable for testing also the sensitivity of detectors, which are used most often for the detection of CWC-related chemicals ⁽²⁴⁾.

A column performance test is used to ensure the proper condition of columns and the stability of retention parameters. Because CWC-related chemicals greatly differ both chemically and physically from each other, the test chemicals have been selected so that their physical, chemical, and retention properties are different, and so that they elute evenly over the whole chromatogram. The use of the following chemicals in a column performance test is recommended: trimethylphosphate, 2,6-dimethylphenol, 5-chloro-2-methylaniline, tri-*n*-butylphosphate, dibenzothiophene, malathion, and methyl stearate. The concentration of test chemicals depends on the sensitivity of detectors. The

columns should be tested at quite a low concentration level, about 10–50 times above the detection limit. The peak shape of trimethylphosphate can be used to estimate the column activity. The acidic and basic properties of the column can be estimated by comparing the peak heights and areas of phenol and aniline peaks. The same mixture is also useful for quality control in GC/MS to check the tuning and the performance of the instrument by checking the correctness of the mass spectra and the isotope ratios.

ABBREVIATIONS AND ACRONYMS

AED	Atomic Emission Detector
CWC	Chemical Weapons Convention
ECD	Electron Capture Detector
EIMS	Electron Impact Mass Spectrometry
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
ID	Internal Diameter
MS	Mass Spectrometry
NPD	Nitrogen–Phosphorus Detector
OCAD	OPCW Central Analytical Database
OPCW	Organization for Prohibition of Chemical Weapons
PID	Photoionization Detector
PFPD	Pulsed Flame Photometric Detector
RIs	Retention Indices
GB	Sarin
GD	Soman
SCD	Sulfur Chemiluminescence Detector
TMS	Trimethylsilyl

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Table 3. Test compounds for checking the sensitivity of FID, NPD, and FPD

Detector	Compound	Concentration (ng μL^{-1})
FID	C ₁₅ -alkane	5
NPD-N	Azobenzene	1
NPD-P, FPD-P	Malathion	0.3
FPD-S	Dibenzothiophene	2

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APPENDIX 1

Retention index values for various CWC-related chemicals on various columns determined against C- and M-series

Compounds	CAS number	C-Series					M-Series		
		I SE-54	II OV-1701	III OV-1	IV SE-54	V OV-1701	VI SE-54	VII SE-54	VIII OV-1701
Methyl methylphosphonofluoridate	353-88-8	586.6 ^a	841.8				238.8 [†]		279.2 ^a
Pinacolyl alcohol	464-07-3	634.2 ^a	828.6						
Trimethyl phosphite	121-45-9	688.8 ^a	804.7				253.8 [†]		242.4 ^a
Ethyl methylphosphonofluoridate	673-97-2	754.9 ^a	913.2				285.6 [†]		353.2
Methyl ethylphosphonofluoridate	665-03-2	802.7	935.5				306.3		377.5
Isopropyl methylphosphonofluoridate (Sarin)	107-44-8	820.2	953.4	791.8	823.8	965.9	329.3		400.0
Ethyl ethylphosphonofluoridate	650-20-4	865.7	1012.6				388.9		454.5
Propyl methylphosphonofluoridate	763-14-4	868.9	1022.1				392.6		464.8
1-Oxa-4-thiacyclohexane	15980-15-1	877.2	969.7	850.7	880.0	964.1	400.0		409.5
Dimethyl methylphosphonate	756-79-6	880.9	1048.4				403.9	398.8	492.8
2-Chloroethyl vinyl sulfide				873.7	903.0	992.1			
Isopropyl ethylphosphonofluoridate	1189-87-3	906.9	1046.9				432.2		491.6
sec-Butyl methylphosphonofluoridate ^b	352-52-3	915.4	1058.9				438.2		504.6
(2-Vinylthio)ethanol		917.9	1061.5	889.6	923.2	1089.2	440.4		507.4
2-Butyl methylphosphonofluoridate	3090-56-0							443.9	
Triethyl phosphite	122-52-1	921.9	985.3				446.9		427.2
Isobutyl methylphosphonofluoridate	2053-81-8							460.5	
3-Hydroxy-1-methylpiperidine	3554-74-3	947.5	1059.8				471.1		505.4
Ethyl methyl methylphosphonate	18755-36-7	951.5	1112.1				474.9		561.6
Diethyl phosphonate	762-04-9	952.9	1115.0				477.6		565.5
Propyl ethylphosphonofluoridate	2992-95-2	967.1	1112.1				491.3		562.3
Butyl methylphosphonofluoridate	352-63-6	971.2	1121.7				495.0		572.7
Neopentyl methylphosphonofluoridate				948.3	984.0	1107.5		499.1	
Bis(2-chloroethyl) ether									

4-Hydroxy-1-methylpiperidine	106-52-5	987.5	1123.7			510.3	574.7
Isopropyl methyl methylphosphonate	690-64-2	988.6	1137.1			512.1	588.6
Diethyl methylphosphonate	867-17-4			974.9	1006.5		
<i>sec</i> -Butyl ethylphosphonofluoridate	162085-83-8	1015.0	1150.1		1186.9	540.5	603.4
Diethyl methylphosphonate	683-08-9	1015.1	1169.6			544.5	625.5
Isobutyl ethylphosphonofluoridate	2261-83-8	1027.7	1168.0			554.3	623.1
Pinacolyl ^b methylphosphonofluoridate	96-64-0	1043.7	1183.5	1008.1	1045.2	571.9	640.3
(Soman)		1048.1	1189.4	1012.9	1049.3	576.8	646.7
Diethyl phosphorocyanidate	2942-58-7	1046.4	1236.8			574.1	695.9
4-Methyl-2-pentyl methylphosphonofluoridate ^b						580.4	
Dimethyl ethylphosphonate	6163-76-3					588.8	
Butyl ethylphosphonofluoridate	18358-34-4	1067.2	1210.0				582.3
1,4-Dithiacyclohexane	505-29-3	1067.6	1169.2			597.8	668.6
Methyl <i>N,N</i> -dimethylphosphor- amidocyanidate	63815-56-5	1070.2	1281.7	1018.7	1060.2	596.8	623.9
Diisopropyl methylphosphonate	1445-75-6	1072.7	1207.2			600.0	740.7
Pentyl methylphosphonofluoridate	13454-59-6	1073.4	1223.3				
2-Chlorovinylchloroarsine (Lewisite 1)	541-25-3	1082.9	^c			610.0	^c
Bis(2-chloroethyl)methylamine (HN-2)	51-75-2	1087.2	1204.3			618.3	661.9
2-(Diisopropylamino)ethanethiol	5842-07-9			1098.2	1113.4		
Dimethyl isopropylphosphonate							623.3
Cyclopentyl methylphosphonofluoridate	7284-82-4						648.4
Isopropyl trimethylsilyl methylphosphonate		1115.8	1244.6			649.1	701.6
<i>O</i> -Ethyl <i>S</i> -methyl methylphosphonothiolate	51865-09-9	1120.9	1289.8			655.1	749.1

(continued overleaf)

(continued)

Compounds	CAS number	C-Series					M-Series		
		I SE-54	II OV-1701	III OV-1	IV SE-54	V OV-1701	VI SE-54	VII SE-54	VIII OV-1701
2-Chlorobenzaldehyde	89-98-5	1131.1	1273.1				667.3		731.8
<i>O</i> -Ethyl <i>N,N</i> -dimethyl-phosphoramidocyanidate (Tabun)	77-81-6	1133.4	1342.4	1077.9	1131.6	1339.8	668.1		802.8
Diethyl <i>N,N</i> -dimethylphosphoramidate	2404-03-7	1133.5	1277.5				668.6		737.9
Triethyl phosphate	78-40-0			1090.5	1137.2	1308.5			
<i>O</i> -Ethyl <i>O</i> -trimethylsilyl methylphosphonothiolate		1137.7	1239.6				672.6		697.9
Pinacolyl ethylphosphonofluoridate	97931-20-9	1141.4	1279.7				677.4		739.4
Diethyl dimethylphosphoramidate	2404-03-7			1096.6	1145.2	1295.6			
Methyl neopentyl methylphosphonate								680.4	
Bis(trimethylsilyl) methylphosphonate	18279-83-9	1154.1	1276.1				689.8		733.9
Bis(2-chloroethyl)ethylamine (HN-1)	538-07-8	1156.1	1273.6				692.2		732.5
Dimethyl propylphosphonate	18755-43-6							696.7 696.9	
3-Methyl-1-butyl methyl methylphosphonate									
Isopropyl <i>N,N</i> -dimethyl-phosphoramidocyanidate	63815-55-4	1161.0	1360.9				697.3		823.4
Pentyl ethylphosphonofluoridate	162085-84-9	1165.2	1313.5				702.7		773.9
Ethyl isopropyl <i>N,N</i> -dimethyl-phosphoramidate	99520-56-6			1121.4	1166.6	1308.5			
2-(Diisopropylamino)ethyl trimethylsilyl ether		1170.6	1186.8				708.0		644.7
Hexyl methylphosphonofluoridate	113548-89-3	1171.5	1326.5				709.5		787.1
1,4,5-Oxadithiepane	3886-40-6	1172.0	1306.3	1117.8	1162.7	1289.9	709.6		766.1
Diethyl ethylphosphonate	78-38-6							709.7	
<i>O,S</i> -Diethyl methylphosphonothiolate	25111-10-6	1174.3	1334.5				716.0		796.0

Bis(2-chloroethyl)sulfide (Mustard gas)	505-60-2	1177.9	1337.4	1123.8	1172.7	1325.8	715.5	797.3
2-Pentyl methyl methylphosphonate							716.5	
Hemisulfur mustard	693-30-1			1132.3	1177.5	1400.0		
Thiodiglycol	111-48-8	1184.4	1467.6	1130.9 1169.2	1181.5 1200.0	1458.2	723.4	934.0
(2-Chloroethylthio)ethyl ether								
Dipropyl methylphosphonate	6410-56-6	1200.0	1350.4				740.9	811.8
Methyl pinacolyl	7040-59-7	1208.3	1360.2				748.7	822.7
methylphosphonate ^b		1207.3	1407.5				747.7	874.5
Phenyl	133826-40-1							
methylphosphonofluoridate							738.5	
Diisopropyl ethylphosphonate	1067-69-2							
Diethyl isopropylphosphonate								
4-Methyl-1-butyl								
methyl methylphosphonate							748.3	
2-Chloroethyl 3-chloropropyl				1168.8	1212.6	1357.9	748.5	
sulfide							754.6	
Ethyl tetramethylphosphoro-	2404-65-1			1158.7	1216.6	1392.8		
diamide								
4-Ethyl-2,6,7-trioxa-1-phospha-	824-11-3	1221.1	1415.7				761.2	882.7
bicyclo[2.2.2]octane								
Propyl <i>N,N</i> -dimethylphosphor-	162085-86-1	1222.1	1432.7				761.4	899.4
amidoacyanide								
Tetramethylphosphorodiamidic								
cyanide				1179.5	1245.7	1514.7		
(2-Chloroethylthio)ethyl				1185.3	1222.8	1354.1		
vinyl ether							787.1	
Diisopropyl								
isopropylphosphonate							787.3	
Diethyl propylphosphonate								
Cyclohexyl	18812-51-6							
methylphosphonothiono-	4241-34-3	1249.6	1365.8				788.2	828.4
fluoridate								
<i>sec</i> -Butyl <i>N,N</i> -dimethyl-		1256.1	1457.8				794.9	924.4
phosphoramidocyanide ^b		1259.3	1461.3				798.3	927.9
Neopentyl trimethylsilyl								
methylphosphonate							795.9	

(continued overleaf)

(continued)

Compounds	CAS number	C-Series					M-Series		
		I SE-54	II OV-1701	III OV-1	IV SE-54	V OV-1701	VI SE-54	VII SE-54	VIII OV-1701
2-Methylcyclohexyl methylphosphonofluoridate ^b	85473-32-1	1260.2	1422.5	1210.2	1256.7	1421.1	798.0		889.7
Hexyl ethylphosphonofluoridate	35445-19-1	1261.7	1425.5	1211.7	1258.5	1423.3	800.0		892.6
O-Trimethylsilyl-3-quinuclidinol		1265.3	1413.7				805.0		881.0
3-Methyl-1-butyl trimethylsilyl methylphosphonate		1268.0	1332.1				808.2	808.3	793.2
Heptyl methylphosphonofluoridate	162085-82-7	1272.4	1427.8						
Isobutyl N,N-dimethylphosphor- amidocyanidate	162085-88-3	1272.9	1480.9				812.8	817.8	895.2
2-Pentyl trimethylsilyl methylphosphonate							812.2		947.4
2-(Diisopropylamino)ethyl ethyl sulfide	110501-54-7			1263.9	1277.6	1333.9		822.6	
Bis(2-chloropropyl)sulfide	22535-54-2			1235.8	1275.6	1420.5			951.1
Chloroacetophenone (CN)	532-27-4	1290.1	1484.5	1230.2	1283.0	1471.0	833.2	821.3	^c
Diisopropyl propylphosphonate									
Lewisite 2	40334-69-8	1289.5	^c				829.9	842.6	
Diisobutyl methylphosphonate		1303.6	1499.3				845.9		965.9
Phenyl ethylphosphonofluoridate		1307.1	1471.9				849.9		939.0
Cyclohexyl ethylphosphonofluoridate	7284-84-6								
Methyl cyclopentyl methylphosphonate								825.0	
Methylbis[2- (trimethylsiloxy)ethyl]- amine	76710-52-6	1314.2	1344.7				857.6		807.0
Butyl N,N-dimethyl- phosphoramidocyanidate	162085-87-2	1317.0	1530.1				859.7		996.5
2-Chloroethyl 4-chlorobutyl sulfide				1269.3	1312.3	1457.1			
2-Ethanol 3-propanol sulfide	5323-60-4	1319.6	1593.5				862.0		1063.0
Pinacolyl trimethylsilyl methylphosphonate		1319.7	1448.6				861.7		914.8
4-Methyl-2-pentyl trimethylsilyl methylphosphonate								864.3	
4-Isopropyl-2,6,7-trioxa-1- phosphabicyclo[2.2.2]octane	51486-55-6	1327.2	1530.5				871.2		997.2

(continued)

Compounds	C-Series					M-Series			
	CAS number	I SE-54	II OV-1701	III OV-1	IV SE-54	V OV-1701	VI SE-54	VII SE-54	VIII OV-1701
Bis[2(vinylthio)ethyl]ether	114811-39-1			1425.6	1466.4	1600.0	1062.9		1205.1
Hexyl <i>N,N</i> -dimethyl- phosphoramidocyanidate	162085-90-7	1512.9	1733.9						
Dipinacolyl methylphosphonate	7040-58-6							1065.0	
<i>O</i> -Ethyl	98543-25-0	1521.7	1691.6				1072.1		1162.8
<i>S</i> -2-(dimethylamino)ethyl ethylphosphonothiolate									
Diisobutyl propylphosphonate	2404-58-2							1075.0 1083.3	
Dibutyl ethylphosphonate	162085-92-9	1532.4	1689.2				1083.6		1160.6
<i>O</i> -Isopropyl									
<i>S</i> -2-(dimethylamino)ethyl ethylphosphonothiolate									
Dibutyl isopropylphosphonate	919-21-1	1557.5	1744.9				1110.2	1108.3	1217.0
<i>O</i> -Ethyl									
<i>S</i> -3-(dimethylamino)propyl methylphosphonothiolate									
2-Chlorobenzalmononitrile (CS)	2698-41-1	1564.0	1823.9	1489.6	1555.1	1805.6	1116.6		1299.3
Bis(2-chloroethylthio)methane	63869-13-6	1569.2	1789.6				1121.9		1264.6
Bis(2-hydroxyethylthio)methane	44860-68-6	1573.9	1921.7				1127.8		1395.8
Di- <i>n</i> -amyl methylphosphonate								1132.4	
<i>O</i> -Ethyl <i>S</i> -2-(diethylamino)ethyl methylphosphonothiolate	21770-86-5	1594.5	1767.9				1148.9		1241.8
Heptyl <i>N,N</i> -dimethyl- phosphoramidocyanidate	162085-91-8	1610.9	1837.3				1165.4		1313.0
Dibutyl propylphosphonate								1175.0	
<i>O</i> -Methyl	170800-77-8	1621.7	1790.1				1175.5		1266.0
<i>S</i> -2-(diethylamino)ethyl ethylphosphonothiolate									
Bis(2-chloroethyl) trisulfide				1561.7	1626.3	1805.4		1184.8	
4-Nitro-2,6,7-trioxa-1-phospha- bicyclo[2.2.2]octane-1-oxide	505-60-2								
Bis(2-trimethylsiloxyethylthio) disulfide		1630.7	1714.8				1184.9		1187.2
<i>O</i> -Ethyl <i>S</i> -2-(ethylthio)ethyl methylphosphonothiolate	556-75-2	1632.1	1848.2				1187.3		1325.2
Benzophenone	119-61-9	1644.5	1827.2				1200.0		1303.0

Tris[2-(trimethylsiloxy)ethyl]amine	20836-42-4	1645.1	1672.0			1200.0	1144.7
Diphenylmethanol	91-01-0	1651.7	1868.3			1207.4	1343.5
<i>O</i> -Ethyl <i>S</i> -2-(diethylamino)ethyl ethylphosphonothiolate	21738-25-0	1670.9	1832.3			1224.7	1308.1
<i>O</i> -Isopropyl <i>S</i> -2-(diethylamino)ethyl ethylphosphonothiolate		1680.0	1826.9			1234.0	1302.9
1,2-Bis(2-hydroxyethylthio)ethane	5244-34-8	1702.8	2066.3			1258.6	1543.7
1,2-Bis(2-chloroethylthio)ethane (Sesquimustard)	3563-36-8	1703.3	1945.3	1622.7	1688.8	1257.6	1419.5
4-Methyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane-1-oxide							1268.9
<i>O</i> -Ethyl <i>S</i> -2-(diisopropylamino)ethyl methylphosphonothiolate (VX)	50782-69-9	1713.0	1882.2	1664.1	1710.1	1271.1	1359.4
(2-Chloroethylthio)ethyl (vinylthio)ethyl ether				1660.6	1714.1		1907.6
(2-Hydroxyethylthio)ethyl (vinylthio)ethyl ether				1660.5	1716.6		1971.5
<i>O</i> -Methyl <i>S</i> -2-(diisopropylamino)ethyl ethylphosphonothioate	162085-94-1	1741.0	1905.8			1298.0	1380.2
1,7-Dioxo-4,10-dithiacyclododecane	294-95-1	1757.0	1965.4	1681.0	1742.9	1314.7	1441.4
Bis(2-trimethylsiloxyethylthio)-methane		1767.1	1870.2			1325.4	1347.2
Di- <i>n</i> -hexyl methylphosphonate							1330.9
<i>O</i> -Isopropyl <i>S</i> -2-(diisopropylamino)ethyl ethylphosphonothioate	162085-95-1	1789.3	1933.7			1348.6	1408.4
<i>S</i> -Ethyl <i>S</i> -[2-(diisopropylamino)ethyl]methylphosphonothiolate	110501-55-8			1759.8	1793.1	1934.1	
1,3-Bis(2-hydroxyethylthio)propane	16260-48-3	1809.2	2170.8			1368.8	1649.9
1,3-Bis(2-chloroethylthio)propane	63905-10-2	1810.9	2053.7			1369.4	1530.7
Dibenz(b,f)1,4-oxazepin (CR)	257-07-8	1810.9	2017.3				1495.1
4-Ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane-1-oxide	1005-93-2	1827.9	2486.6	1736.0	1800.0	1369.5	1971.0
Bis[2-(diisopropylamino)ethyl] sulfide	6006-58-2			1820.4	1836.1	1388.3	1404.1
							1902.6

(continued overleaf)

(continued)

Compounds	C-Series					M-Series			
	CAS number	I SE-54	II OV-1701	III OV-1	IV SE-54	V OV-1701	VI SE-54	VII SE-54	VIII OV-1701
Methyl benzoate	76-89-1	1853.2	2058.5				1410.6		1536.6
<i>O</i> -Ethyl <i>S</i> -nonyl methyl- <i>l</i> -phosphonothiolate	13088-89-6	1871.9	2047.9				1431.9		1527.7
1,2-Bis(2- trimethylsiloxyethylthio)- ethane		1885.1	2000.0				1444.6		1480.3
Dicyclohexyl methylphosphonate	7040-53-1							1441.6	1774.3
1,4-Bis(2- hydroxyethylthio)butane	7425-93-6	1928.6	2295.6				1498.9		
1,4-Bis(2-chloroethylthio)butane									
4-Propyl-2,6,7-trioxa-1-phospha- bicyclo[2,2,2]octane-1-oxide	142868-93-7	1932.7	2182.8				1492.8	1509.3	1661.7
4-Isopropyl-2,6,7-trioxa-1- phospha-bicyclo[2,2,2]octane- 1-oxide	51052-72-3	1947.4	2609.8				1517.1	1520.2	2090.3
1-Oxa-4,7,10- trithiacyclododecane	40254-02-2			1884.9	1959.9	2190.8			
Bis[(2-chloroethylthio)ethyl] ether	63918-89-8	1990.3	2263.4				1552.7		1742.3
(<i>O</i> -Mustard)				1909.9	1982.6	2241.3			
1,3-Bis(2- trimethylsiloxyethylthio)- propane		1990.5	2111.6				1552.9		1588.3
4-Tertbutyl-2,6,7-trioxa-1- phospha- bicyclo[2,2,2]octane-1-oxide								1608.6	
4-Butyl-2,6,7-trioxa-1-phospha- bicyclo[2,2,2]octane-1-oxide									
Bis[2-(diisopropylamino)ethyl] disulfide				2036.0	2057.9	2146.0			
1,4-Bis(2- trimethylsiloxyethylthio)- butane		2103.2	2228.2				1664.9		1709.2
4-Pentyl-2,6,7-trioxa-1-phospha- bicyclo[2,2,2]octane-1-oxide								1726.9	
Bis[(2-chloroethylthio)ethyl] sulfide				2140.7	2222.0	2543.2			

3,9,12-Trithia-6-oxa-14chloro-1-tetradecane				2160.6	2234.3	2517.3	
1,2-Bis[2-(diisopropylamino)-ethylthio] ethane	110501-58-1			2309.8	2338.7	2460.9	
1,7-Dioxo-4,10,13-trithiacyclopentadecane	52559-77-0			2171.7	2257.1	2535.5	
1-Methyl-3-piperidyl cyclo-pentylphenylglycolate	7121-51-9	2279.8	2456.9 ^a			1845.1	1938.1
1,14-Dichloro-3,9-dithia-6,12-dioxatetradecane				2200.0	2280.4	2577.8	
1-Methyl-4-piperidyl cyclopentylphenylglycolate	37830-21-0	2310.4	2490.3 ^a			1876.8	1971.7
4-Piperidyl cyclopentylphenylglycolate		2350.9	2560.0 ^a			1918.7	2060.5
1-Methyl-3-piperidyl cyclo-hexylphenylglycolate	4354-45-4	2374.0	2556.8 ^a			1942.3	2035.5
1,9-Bis(diisopropylamino)-3,4,7-trithianonane				2532.9	2567.5	2717.5	
1-Methyl-3-piperidyl benzilate	3321-80-0	2400.0	2633.3 ^a			1969.3	2113.5
1-Methyl-4-piperidyl cyclo-hexylphenylglycolate	33445-17-9	2408.4 ^a	2593.8 ^a			1976.9	2071.3
1-Methyl-4-piperidyl benzilate	3608-67-1	2431.4 ^a	2664.1 ^a			2000.0	2147.6
4-Piperidyl cyclohexylphenylglycolate		2445.1 ^a	2653.8 ^a			2017.8	2166.0
4-Piperidyl benzilate	25811-48-7	2466.5 ^a	2729.5 ^a			2043.9	2237.0 ^a
1,14-Dichloro-3,16,12-trithia-9-oxatetradecane				2418.8	2514.5	2864.5	
1,16-Dichloro-3,9,15-trithia-6,12-dioxahaptadecane				2711.8	2813.8	3200.0	
3-Quinuclidinyl benzilate (BZ)	6581-06-2	2628.4 ^a	2948.9 ^a			2221.6 ^a	2416.7 ^a
1,10-Dioxo-4,7,13,16-tetrathia-cyclooctadecane	296-40-2	2833.2 ^a	3389.6 ^a			2394.8 ^a	2780.1 ^a

I and VI: Column SE-54, 25 m × 0.32 mm × 0.25 μm; carrier gas He 1.5 mL min⁻¹, temperature program 40 °C (1 min), heating rate 10 °C min⁻¹ to 280 °C for 10 min ⁽²⁴⁾
 I I and VIII: Column OV-1701, 25 m × 0.32 mm × 0.25 μm; carrier gas He 1.5 mL min⁻¹ temperature program 40 °C (1 min), heating rate 10 °C min⁻¹ to 280 °C for 10 min ⁽²⁴⁾
 I I I: Column OV-1, 15 m × 0.32 mm × 0.25 μm; carrier gas He 35 cm s⁻¹, temperature program 50 °C (2 min), heating rate 10 °C min⁻¹ to 28 °C for 10 min ^(29,33,76-78)
 I V: Column SE-54, 15 m × 0.32 mm × 0.25 μm; carrier gas He 35 cm s⁻¹, temperature program 50 °C (2 min), heating rate 10 °C min⁻¹ to 280 °C for 10 min ^(29,33,76-78)
 V: Column OV-1701, 15 m × 0.32 mm × 0.25 μm; carrier gas He 35 cm s⁻¹, temperature program 50 °C (2 min), heating rate 10 °C min⁻¹ to 280 °C for 10 min ^(29,33,76-78)
 VII: Column SE-54, 30 m × 0.25 mm × 0.25 μm; carrier gas He 35 cm s⁻¹, temperature program 60 °C (3 min), heating rate 10 °C min⁻¹ to 280 °C ⁽⁷⁹⁻⁸⁴⁾

^a Extrapolated

^b A diastereometric compound gives two peaks

^c Compound does not elute through column

APPENDIX 2

RIs of OCAD (Reproduced by permission of the Organization for the Prohibition of Chemical Weapons from the OPCW Central Analytical Database.)

Compounds	CAS number	Retention index
Methylphosphonic difluoride	676-99-3	488
Sulfur dichloride	10545-99-0	543
Ethylphosphonic difluoride	753-98-0	563
Isopropylphosphonic difluoride	677-42-9	631
Ethylphosphonous dichloride	1498-40-4	762
Methyl ethylphosphonofluoridate	665-03-2	803
2- <i>N,N</i> -Dimethylaminoethanethiol	108-02-1	813
<i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethanol	2893-43-8	817
Isopropyl methylphosphonofluoridate	107-44-8	820
Isopropylphosphonous dichloride	25235-15-8	837
<i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethyl chloride	54153-16-1	838
Sulfur monochloride	10025-67-9	851
Propylphosphonous dichloride	15573-31-6	854
Propyl methylphosphonofluoridate	763-14-4	869
Ethyl ethylphosphonofluoridate	650-20-4	870
Dimethyl methylphosphonate	756-79-6	881
Methyl propylphosphonofluoridate		882
2- <i>N,N</i> -Diethylaminoethyl chloride	100-35-6	912
Isopropyl ethylphosphonofluoridate	1189-87-3	912
<i>sec</i> -Butyl methylphosphonofluoridate-a	352-52-3	915
<i>sec</i> -Butyl methylphosphonofluoridate-b	352-52-3	918
Triethyl phosphite	122-52-1	922
Ethyl isopropylphosphonofluoridate	1426-08-0	924
Isobutyl methylphosphonofluoridate	2053-81-8	929
Trimethyl phosphate	512-56-1	938
Ethyl methyl methylphosphonate	18755-36-7	952
Diethyl phosphite	762-04-9	953
Ethyl propylphosphonofluoridate		959
Isopropyl isopropylphosphonofluoridate	665-33-8	964
2,2-Dimethylpropyl methylphosphonofluoridate	372-62-3	967
Dimethyl ethylphosphonate	6163-75-3	968
Butyl methylphosphonofluoridate	352-63-6	970
Propyl ethylphosphonofluoridate	2992-95-2	970
Isopropyl methylphosphonochloridate	1445-76-7	975
2- <i>N,N</i> -Diethylaminoethanethiol	100-38-9	978
<i>N</i> -Ethyl- <i>N</i> -methyl-2-aminoethyl trimethylsilyl ether	60322-88-5	983
1,2-Dimethylpropyl methylphosphonofluoridate-a	6154-51-4	986
Isopropyl methyl methylphosphonate	690-64-2	989
1,2-Dimethylpropyl methylphosphonofluoridate-b	6154-51-4	990
<i>N</i> -Ethyl- <i>N</i> -propyl-2-aminoethyl chloride		996
Isopropyl propylphosphonofluoridate	18358-37-7	999
1-Methylbutyl methylphosphonofluoridate-a	761-93-3	1001
1-Methylbutyl methylphosphonofluoridate-b	761-93-3	1005
1-Ethylpropyl methylphosphonofluoridate	66348-71-8	1010
Dimethyl isopropylphosphonate	54552-77-1	1013
Diethyl methylphosphonate	683-08-9	1015
Dimethyl <i>N,N</i> -dimethylphosphoramidate	597-07-9	1016
<i>sec</i> -Butyl ethylphosphonofluoridate	162085-83-8	1016
Propyl isopropylphosphonofluoridate		1019
Methyl trimethylsilyl methylphosphonate		1023
3-Methylbutyl methylphosphonofluoridate	22107-46-6	1027

(continued)

Compounds	CAS number	Retention index
Isobutyl ethylphosphonofluoridate	2261-83-8	1028
2-Methylbutyl methylphosphonofluoridate		1033
Ethyl methyl ethylphosphonate	5301-65-5	1033
1,3-Dimethylbutyl methylphosphonofluoridate-a	352-53-4	1042
Pinacolyl methylphosphonofluoridate-a	96-64-0	1044
<i>O</i> -Methyl <i>S</i> -methyl methylphosphonothiolate	58259-60-2	1045
Pinacolyl methylphosphonofluoridate-b	96-64-0	1048
1,3-Dimethylbutyl methylphosphonofluoridate-b	352-53-4	1050
2- <i>N,N</i> -Diisopropylaminoethyl chloride	96-79-7	1053
Dimethyl propylphosphonate	18755-43-6	1054
2- <i>N,N</i> -Diisopropylaminoethanol	96-80-0	1057
Propyl propylphosphonofluoridate	18358-36-6	1057
2,2-Dimethylpropyl ethylphosphonofluoridate		1060
2-Chloroethylchloromethylsulfide	2625-76-5	1060
<i>sec</i> -Butyl isopropylphosphonofluoridate		1064
Butyl ethylphosphonofluoridate	18358-34-4	1068
Isopropyl methyl ethylphosphonate	141968-53-8	1068
Methyl <i>N,N</i> -dimethylphosphoramidocyanidate	63815-56-5	1070
Diisopropyl methylphosphonate	1445-75-6	1073
Pentyl methylphosphonofluoridate	13454-59-6	1073
2- <i>N,N</i> -Dipropylaminoethanol	3238-75-3	1074
Isobutyl isopropylphosphonofluoridate	333416-40-3	1075
1-Ethyl-2-methylpropyl methylphosphonofluoridate		1076
Ethyl methyl isopropylphosphonate		1076
<i>sec</i> -Butyl methylphosphonochloridate		1076
1,2-Dimethylbutyl methylphosphonofluoridate-a		1078
1,2-Dimethylpropyl ethylphosphonofluoridate		1078
Ethyl trimethylsilyl methylphosphonate	57451-30-6	1081
2- <i>N,N</i> -Dipropylaminoethyl chloride	36716-60-6	1083
2-Chlorovinylchloroarsine	541-25-3	1083
1,2-Dimethylbutyl methylphosphonofluoridate-b		1084
3,3-Dimethylbutyl methylphosphonofluoridate	660-21-9	1084
Bis(2-chloroethyl)methylamine	51-75-2	1087
1,2-Dimethylbutyl methylphosphonofluoridate-c		1088
1-Methylbutyl ethylphosphonofluoridate-a		1088
1-Ethylbutyl methylphosphonofluoridate-a		1090
Dimethyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidate		1092
1-Ethylbutyl methylphosphonofluoridate-b		1093
1-Methylbutyl ethylphosphonofluoridate-b		1093
1-Methylpentyl methylphosphonofluoridate-a	13172-12-8	1093
1,3,3-Trimethylbutyl methylphosphonofluoridate-a	30593-65-8	1094
1-Methylpentyl methylphosphonofluoridate-b	13172-12-8	1100
<i>sec</i> -Butyl propylphosphonofluoridate		1101
Isopropyl methyl isopropylphosphonate	331954-04-2	1104
1,3,3-Trimethylbutyl methylphosphonofluoridate-b	30593-65-8	1106
Cyclopentyl methylphosphonofluoridate	7284-82-4	1106
Diethyl ethylphosphonate	78-38-6	1108
1-Ethylpropyl ethylphosphonofluoridate		1109
2,2-Dimethylpropyl isopropylphosphonofluoridate		1110
Isobutyl propylphosphonofluoridate	333416-26-5	1112
Isopropyl trimethylsilyl methylphosphonate	199116-08-0	1112
Butyl isopropylphosphonofluoridate		1115
<i>S</i> -Ethyl <i>O</i> -methyl methylphosphonothiolate		1116
2,2-Dimethylpropyl methylphosphonochloridate		1116
2-Methylpentyl methylphosphonofluoridate		1119

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Compounds	CAS number	Retention index
2- <i>N,N</i> -Diisopropylaminoethanethiol	5842-07-9	1120
Ethyl methyl propylphosphonate		1120
Propyl ethylphosphonochloridate		1120
<i>O,O</i> -Diisopropyl methylphosphonothionate	66295-45-2	1121
<i>O</i> -Ethyl <i>S</i> -methyl methylphosphonothiolate	51865-09-9	1121
3-Methylbutyl ethylphosphonofluoridate		1121
2-Ethylbutyl methylphosphonofluoridate	126204-48-6	1122
2-Methylbutyl ethylphosphonofluoridate		1124
1-Ethyl-2,2-dimethylpropyl methylphosphonofluoridate-a		1125
1-Ethyl-2,2-dimethylpropyl methylphosphonofluoridate-b		1128
4-Methylpentyl methylphosphonofluoridate		1128
Methyl propyl ethylphosphonate	170082-59-4	1128
1,3-Dimethylbutyl ethylphosphonofluoridate-a		1129
1,2-Dimethylpropyl isopropylphosphonofluoridate-a		1130
<i>O</i> -Methyl <i>S</i> -methyl ethylphosphonothiolate	84044-17-7	1131
1,2-Dimethylpropyl isopropylphosphonofluoridate-b		1131
3-Methylpentyl methylphosphonofluoridate	199850-60-7	1133
Diethyl isopropylphosphonate	1538-69-8	1133
Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate	77-81-6	1133
Diethyl <i>N,N</i> -dimethylphosphoramidate	2404-03-7	1134
2-Methylcyclopentyl methylphosphonofluoridate	193090-50-5	1137
<i>O</i> -Ethyl <i>O</i> -trimethylsilyl methylphosphonothionate		1138
1,3-Dimethylbutyl ethylphosphonofluoridate-b		1138
1,2-Dimethylpropyl methylphosphonochloridate		1139
1-Methylbutyl isopropylphosphonofluoridate-a		1139
Pinacolyl ethylphosphonofluoridate	97931-20-9	1141
1-Isopropyl-2-methylpropyl methylphosphonofluoridate	113548-85-9	1143
1-Methylbutyl isopropylphosphonofluoridate-b		1143
2,2-Dimethylpropyl propylphosphonofluoridate		1143
Cyclopropylmethyl isopropylphosphonofluoridate		1143
Methyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanidate		1145
1-Isopropylbutyl methylphosphonofluoridate-a		1147
2- <i>N,N</i> -Dipropylaminoethanethiol	5842-06-8	1148
2-Methoxyethyl methylphosphonochloridate		1148
Isopropyl methyl propylphosphonate	211923-89-6	1148
1-Isopropylbutyl methylphosphonofluoridate-b		1149
1,4-Dimethylpentyl methylphosphonofluoridate-a	199850-62-9	1151
Bis(trimethylsilyl) methylphosphonate	18279-83-9	1151
1-Ethylpropyl isopropylphosphonofluoridate		1152
Butyl propylphosphonofluoridate		1152
3-Methylcyclopentyl methylphosphonofluoridate	193090-47-0	1154
Diisopropyl ethylphosphonate	1067-69-2	1154
Dimethyl <i>N</i> -isopropyl- <i>N</i> -methylphosphoramidate		1154
Bis(2-chloroethyl)ethylamine	538-07-8	1156
Propyl trimethylsilyl methylphosphonate		1156
1-Methylbutyl methylphosphonochloridate-a		1157
1,4-Dimethylpentyl methylphosphonofluoridate-b	199850-62-9	1158
1-Methylbutyl methylphosphonochloridate-b		1159
<i>sec</i> -Butyl ethylphosphonochloridate		1159
Dimethyl <i>N,N</i> -diethylphosphoramidate	65659-19-0	1161
Isopropyl <i>N,N</i> -dimethylphosphoramidocyanidate	63815-55-4	1161
<i>sec</i> -Butyl methyl ethylphosphonate-a	170082-78-7	1161

(continued)

Compounds	CAS number	Retention index
1,2-Dimethylpropyl propylphosphonofluoride-a		1162
1,2-Dimethylpropyl propylphosphonofluoride-b		1165
1-Ethyl-2-methylpropyl ethylphosphonofluoride		1165
<i>sec</i> -Butyl methyl ethylphosphonate-b	170082-78-7	1165
1-Ethylpropyl methylphosphonochloride		1166
Pentyl ethylphosphonofluoride	162085-84-9	1166
<i>N,N</i> -Diethylphosphoramidic dichloride	1498-54-0	1167
Methyl propyl isopropylphosphonate		1168
3-Methylbutyl isopropylphosphonofluoride		1169
Hexyl methylphosphonofluoride	113548-89-3	1169
1,2-Dimethylbutyl ethylphosphonofluoride-a		1171
1-Methylbutyl propylphosphonofluoride-a		1171
2- <i>N,N</i> -Diisopropylaminoethyl trimethylsilyl ether		1171
2-Methylbutyl isopropylphosphonofluoride		1171
<i>O</i> -Methyl <i>S</i> -methyl isopropylphosphonothiolate		1173
Diethyl propylphosphonate	18812-51-6	1173
<i>O</i> -Ethyl <i>S</i> -ethyl methylphosphonothiolate	2511-10-6	1174
3,3-Dimethylbutyl ethylphosphonofluoride		1174
Isobutyl ethylphosphonochloride		1174
1-Ethylpentyl methylphosphonofluoride-a		1175
Propyl isopropylphosphonochloride	13242-70-1	1175
1-Ethylbutyl ethylphosphonofluoride-a		1176
1,2-Dimethylbutyl ethylphosphonofluoride-b		1177
1,3-Dimethylbutyl isopropylphosphonofluoride-a		1177
1-Methylbutyl propylphosphonofluoride-b		1178
Bis(2-chloroethyl)sulfide	505-60-2	1178
1-Methylpentyl ethylphosphonofluoride-a		1179
1-Ethylpentyl methylphosphonofluoride-b		1180
Dimethyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidate		1180
1,2-Dimethylbutyl ethylphosphonofluoride-c		1181
1-Ethylbutyl ethylphosphonofluoride-b		1181
3-Methylbutyl methylphosphonochloride		1183
Isobutyl methyl ethylphosphonate	170082-79-8	1183
1-Methylhexyl methylphosphonofluoride-a	674-95-3	1184
Bis(2-hydroxyethyl)sulfide	111-48-8	1184
Isopropyl trimethylsilyl ethylphosphonate		1184
<i>sec</i> -Butyl trimethylsilyl methylphosphonate		1185
1,3-Dimethylbutyl isopropylphosphonofluoride-b		1186
2-Methylbutyl methylphosphonochloride		1186
Diisopropyl isopropylphosphonate	3759-39-5	1186
Pinacolyl isopropylphosphonofluoride-a		1186
1-Ethylpropyl propylphosphonofluoride		1187
1-Methylpentyl ethylphosphonofluoride-b		1187
1,3,3-Trimethylbutyl ethylphosphonofluoride-a		1188
Pinacolyl isopropylphosphonofluoride-b		1189
1-Methylhexyl methylphosphonofluoride-b	674-95-3	1191
2- <i>N,N</i> -Dipropylaminoethyl trimethylsilyl ether		1193
<i>sec</i> -Butyl methyl isopropylphosphonate-a		1196
Cyclopentyl ethylphosphonofluoride		1197
Diethyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidate		1197
1,3,3-Trimethylbutyl ethylphosphonofluoride-b		1199
<i>sec</i> -Butyl methyl isopropylphosphonate-b		1200
Pinacolyl methylphosphonochloride	7040-57-5	1203
Ethyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanide		1204
Isobutyl trimethylsilyl methylphosphonate	199116-09-1	1204

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Compounds	CAS number	Retention index
Methyl pinacolyl methylphosphonate	7040-59-7	1204
2-Methylbutyl propylphosphonofluoride		1206
Phenyl methylphosphonofluoride	133826-40-1	1207
1,3-Dimethylbutyl propylphosphonofluoride-a		1208
2,2-Dimethylpropyl ethylphosphonochloride		1208
2-Ethylbutyl ethylphosphonofluoride		1208
Cyclohexyl methylphosphonofluoride	329-99-7	1208
Methyl propyl propylphosphonate	18755-44-7	1208
Cyclopentylmethyl methylphosphonofluoride		1209
Pentyl isopropylphosphonofluoride		1210
2-Methylpentyl ethylphosphonofluoride		1211
2,3-Dimethylpentyl methylphosphonofluoride		1212
Ethyl methyl <i>N,N</i> -diethylphosphoramidate	89893-77-6	1212
Pinacolyl propylphosphonofluoride-a		1212
1-Ethyl-2-methylpropyl isopropylphosphonofluoride-a		1214
<i>O</i> -Methyl <i>S</i> -methyl propylphosphonothiolate	90220-19-2	1215
Pinacolyl propylphosphonofluoride-b		1215
Isopropyl trimethylsilyl isopropylphosphonate	111204-34-3	1216
1-Ethyl-2-methylpropyl isopropylphosphonofluoride-b		1217
Butyl ethylphosphonochloride		1217
1-Ethyl-2,2-dimethylpropyl ethylphosphonofluoride-a		1218
Bis(trimethylsilyl) ethylphosphonate	1641-57-2	1218
Isobutyl methyl isopropylphosphonate		1218
1-Ethyl-2,2-dimethylpropyl ethylphosphonofluoride-b		1220
1,3-Dimethylbutyl propylphosphonofluoride-b		1221
1,2-Dimethylbutyl isopropylphosphonofluoride-a		1222
Butyl methyl ethylphosphonate	170082-77-6	1222
Diisopropyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidate		1222
Propyl <i>N,N</i> -dimethylphosphoramidocyanide	162085-86-1	1222
2,4,4-Trimethylpentyl methylphosphonofluoride		1223
4-Methylpentyl ethylphosphonofluoride		1223
3-Methylpentyl ethylphosphonofluoride		1224
Diisopropyl propylphosphonate	18812-55-0	1224
3,3-Dimethylbutyl isopropylphosphonofluoride		1225
1,2-Dimethylbutyl methyl methylphosphonate-a		1226
1,2-Dimethylbutyl isopropylphosphonofluoride-b		1227
1-Ethylbutyl isopropylphosphonofluoride		1227
1-Methylpentyl isopropylphosphonofluoride-a		1228
2-Methylcyclopentyl ethylphosphonofluoride-a		1229
Pentyl methylphosphonochloride	53864-20-3	1229
1,2-Dimethylbutyl isopropylphosphonofluoride-c		1230
1,2-Dimethylbutyl methyl methylphosphonate-b		1230
Methyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanide		1230
1,2-Dimethylpropyl ethylphosphonochloride		1231
1-Ethyl-2-methylpentyl methylphosphonofluoride-a		1232
2-Methylcyclopentyl ethylphosphonofluoride-b		1232
1-Isopropyl-2-methylpropyl ethylphosphonofluoride		1234
2,2-Dimethylpropyl trimethylsilyl methylphosphonate	214042-77-0	1234
1,2-Dimethylbutyl methyl methylphosphonate-c		1235
1-Methylpentyl isopropylphosphonofluoride-b		1235
1-Isopropylbutyl ethylphosphonofluoride-a		1236

(continued)

Compounds	CAS number	Retention index
1,4-Dimethylpentyl ethylphosphonofluoride-a		1237
1-Ethyl-2-methylpentyl methylphosphonofluoride-b		1240
1,3,3-Trimethylbutyl isopropylphosphonofluoride-a		1241
3-Methylcyclohexyl methylphosphonofluoride-a	113548-86-0	1241
1-Isopropylbutyl ethylphosphonofluoride-b		1242
1-Methylbutyl ethylphosphonochloride-a		1242
<i>sec</i> -Butyl methyl propylphosphonate-a		1242
1-Ethyl-2-methylpentyl methylphosphonofluoride-c		1243
1-Isobutyl-3-methylbutyl methylphosphonofluoride	193090-16-3	1243
Cyclopentyl isopropylphosphonofluoride		1243
Isopropyl 2-methoxyethyl methylphosphonate	192446-28-9	1244
<i>sec</i> -Butyl methyl propylphosphonate-b		1244
1-Methylbutyl ethylphosphonochloride-b		1245
Butyl trimethylsilyl methylphosphonate		1245
<i>tert</i> -Butyldimethylsilyl methyl methylphosphonate		1246
1,4-Dimethylpentyl ethylphosphonofluoride-b		1247
1-Ethyl-2-methylpropyl propylphosphonofluoride		1247
Pentyl propylphosphonofluoride		1247
1,5-Dimethylhexyl methylphosphonofluoride-a	159395-76-3	1248
1-Ethyl-2-methylbutyl ethylphosphonofluoride-a		1248
3-Methylcyclopentyl ethylphosphonofluoride		1248
1,2-Dimethylpropyl trimethylsilyl methylphosphonate		1249
1,3,3-Trimethylbutyl isopropylphosphonofluoride-b		1249
<i>O</i> -Cyclohexyl methylphosphonothionofluoride	4241-34-3	1250
Diethyl <i>N</i> -isopropyl- <i>N</i> -methylphosphoramidate		1250
Methyl 1,3,3-trimethylbutyl methylphosphonate-a		1250
<i>O</i> -Isopropyl <i>S</i> -methyl isopropylphosphonothiolate		1251
1-Ethylpropyl ethylphosphonochloride		1252
Isopropyl trimethylsilyl propylphosphonate		1252
1,2-Dimethylbutyl propylphosphonofluoride-a		1253
Methyl 1,3,3-trimethylbutyl methylphosphonate-b		1253
1-Ethyl-2-methylbutyl ethylphosphonofluoride-b		1254
4-Methylcyclohexyl methylphosphonofluoride-a	113548-87-1	1254
Bis(trimethylsilyl) isopropylphosphonate		1254
2-Methylcyclohexyl methylphosphonofluoride-a	85473-32-1	1255
1-Ethylbutyl methylphosphonochloride		1256
1-Methylpentyl methylphosphonochloride-a		1256
2-Ethylbutyl isopropylphosphonofluoride		1256
<i>sec</i> -Butyl <i>N,N</i> -dimethylphosphoramidocyanide-a	162085-89-4	1256
1-Propylpentyl methylphosphonofluoride-a		1257
2-Methylcyclohexyl methylphosphonofluoride-b	85473-32-1	1257
3-Methylcyclohexyl methylphosphonofluoride-b	113548-86-0	1257
1-Ethylbutyl propylphosphonofluoride-a		1258
2,2-Dimethylhexyl methylphosphonofluoride	19447-69-9	1258
1,2-Dimethylbutyl propylphosphonofluoride-b		1259
1,5-Dimethylhexyl methylphosphonofluoride-b	159395-76-3	1259
Butyl methyl isopropylphosphonate		1259
<i>sec</i> -Butyl <i>N,N</i> -dimethylphosphoramidocyanide-b	162085-89-4	1259
1-Methylpentyl methylphosphonochloride-b		1260
1-Methylpentyl propylphosphonofluoride-a		1260
2-Methylpentyl isopropylphosphonofluoride	333416-41-4	1260
Bis(<i>sec</i> -butyl) methylphosphonate	22668-63-9	1260
1,2-Dimethylbutyl propylphosphonofluoride-c		1261
1-Propylpentyl methylphosphonofluoride-b		1261
3,3-Dimethylbutyl propylphosphonofluoride		1262

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Compounds	CAS number	Retention index
<i>O</i> -Methyl <i>O</i> -propyl propylphosphonothionate		1263
1-Methylbutyl trimethylsilyl methylphosphonate	214042-69-0	1263
Diethyl <i>N,N</i> -diethylphosphoramidate	3167-69-9	1263
Isobutyl methyl propylphosphonate		1263
1-Ethylbutyl propylphosphonofluoride-b		1264
1,2-Dimethylbutyl propylphosphonofluoride-d		1265
Hexyl ethylphosphonofluoride	135445-19-1	1265
1-Ethyl-2,2-dimethylpropyl isopropylphosphonofluoride-a		1266
1-Ethyl-2,2-dimethylpropyl methyl methylphosphonate-a		1266
1-Ethylpentyl ethylphosphonofluoride-a		1266
1,3,3-Trimethylbutyl propylphosphonofluoride-a		1267
1-Ethylpropyl trimethylsilyl methylphosphonate		1268
3-Quinuclidinyl trimethylsilyl ether		1268
4-Methylcyclohexyl methylphosphonofluoride-b	113548-87-1	1268
4-Methylpentyl isopropylphosphonofluoride		1268
3-Methylpentyl isopropylphosphonofluoride		1269
Cyclopentyl methylphosphonochloride		1270
1,3-Dimethylbutyl methyl ethylphosphonate		1271
Dipropyl ethylphosphonate	6163-76-4	1271
1-Methylpentyl propylphosphonofluoride-b		1272
Heptyl methylphosphonofluoride	162085-82-7	1272
1-Ethyl-2,2-dimethylpropyl isopropylphosphonofluoride-b		1273
1-Ethyl-2,2-dimethylpropyl methyl methylphosphonate-b		1273
1-Ethylpentyl ethylphosphonofluoride-b		1273
Isobutyl <i>N,N</i> -dimethylphosphoramidocyanide	162085-88-3	1273
1-Methylhexyl ethylphosphonofluoride-a		1274
3-Methylbutyl ethylphosphonochloride		1274
Ethyl <i>N,N</i> -diethylphosphoramidocyanide	63815-60-1	1274
3,5-Dimethylcyclohexyl methylphosphonofluoride-a		1276
2-Methylbutyl ethylphosphonochloride		1277
2-Methylcyclopentyl isopropylphosphonofluoride-a		1277
Diisopropyl <i>N</i> -isopropyl- <i>N</i> -methylphosphoramidate		1277
Propyl trimethylsilyl isopropylphosphonate		1277
2-Propylpentyl methylphosphonofluoride		1278
Methyl 3-methylbutyl ethylphosphonate		1278
1,2-Dimethylbutyl ethyl methylphosphonate-a		1279
1-Isopropyl-2-methylpropyl methyl methylphosphonate		1279
2-Methylcyclopentyl isopropylphosphonofluoride-b		1279
1,3,3-Trimethylbutyl propylphosphonofluoride-b		1281
1,2-Dimethylbutyl ethyl methylphosphonate-b		1282
1-Isopropylbutyl isopropylphosphonofluoride-a		1283
<i>O</i> -Isopropyl <i>O</i> -trimethylsilyl isopropylphosphonothionate		1284
1-Isopropyl-2-methylpropyl isopropylphosphonofluoride		1284
1-Methylhexyl ethylphosphonofluoride-b		1284
1,2-Dimethylbutyl ethyl methylphosphonate-c		1285
1,4-Dimethylpentyl isopropylphosphonofluoride-a		1285
Cyclopentyl propylphosphonofluoride		1285
Bis(trimethylsilyl) propylphosphonate		1286

(continued)

Compounds	CAS number	Retention index
Diethyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidate		1286
3,5-Dimethylcyclohexyl methylphosphonofluoridate-ar		1287
1-Isopropylbutyl methyl methylphosphonate-a		1289
1,2-Dimethylbutyl ethyl methylphosphonate-d		1290
Bis(2-chlorovinyl)chloroarsine	40334-69-8	1290
2-Ethylbutyl propylphosphonofluoridate		1291
3-Methylcyclopentyl isopropylphosphonofluoridate-a		1291
Cyclopropylmethyl methyl isopropylphosphonate		1291
1-Isopropylbutyl isopropylphosphonofluoridate-b		1292
1-Isopropylbutyl methyl methylphosphonate-b		1292
1-Ethyl-2-methylpropyl methyl ethylphosphonate-a		1293
1,4-Dimethylpentyl isopropylphosphonofluoridate-b		1294
3-Methylcyclopentyl isopropylphosphonofluoridate-b		1294
2-Methylpentyl propylphosphonofluoridate	333416-27-6	1295
Ethyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanidate		1295
Methyl 2-methylcyclopentyl methylphosphonate		1295
Propyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanidate		1296
1-Ethyl-2-methylpropyl methyl ethylphosphonate-b		1297
<i>O</i> -Isopropyl <i>S</i> -methyl propylphosphonothiolate		1298
2-Methylbutyl trimethylsilyl methylphosphonate		1298
3-Methylbutyl trimethylsilyl methylphosphonate	214042-71-4	1298
1,4-Dimethylpentyl methyl methylphosphonate-a		1299
1-Ethyl-2,2-dimethylpropyl propylphosphonofluoridate		1300
<i>tert</i> -Butyldimethylsilyl ethyl methylphosphonate	126281-75-2	1300
Ethyl 1,3,3-trimethylbutyl methylphosphonate-a		1301
1,4-Dimethylpentyl methyl methylphosphonate-b		1302
2-Ethylhexyl methylphosphonofluoridate	458-71-9	1302
4-Methylpentyl propylphosphonofluoridate		1303
Diisopropyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidate		1303
3-Methylpentyl propylphosphonofluoridate		1304
Phenyl ethylphosphonofluoridate		1304
1,2-Dimethylbutyl methyl ethylphosphonate-a		1305
Butyl methyl propylphosphonate		1305
Cyclohexyl ethylphosphonofluoridate	7284-84-6	1306
Cyclopentylmethyl ethylphosphonofluoridate		1306
3,5-Dimethylcyclohexyl methylphosphonofluoridate-b		1307
Hexyl isopropylphosphonofluoridate		1308
1,2-Dimethylbutyl methyl ethylphosphonate-b		1309
Ethyl 1,3,3-trimethylbutyl methylphosphonate-b		1309
2,4,4-Trimethylpentyl ethylphosphonofluoridate		1310
Methyl 3-methylcyclopentyl methylphosphonate		1310
Methyl pinacolyl isopropylphosphonate-a		1310
Cyclohexylmethyl methylphosphonofluoridate		1311
Methyl <i>N,N</i> -diisopropylphosphoramidocyanidate		1312
<i>O</i> -Isopropyl <i>O</i> -trimethylsilyl propylphosphonothionate		1313
1-Ethylbutyl methyl ethylphosphonate		1313
Methyl 3-methylbutyl isopropylphosphonate		1313
1-Ethylpentyl isopropylphosphonofluoridate-a		1314
1-Isopropyl-2-methylpropyl propylphosphonofluoridate		1314
1-Isopropylbutyl propylphosphonofluoridate-a		1314
2,6-Dimethylcyclohexyl methylphosphonofluoridate		1314

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Compounds	CAS number	Retention index
3,5-Dimethylcyclohexyl methylphosphonofluoride-c		1314
Bis(2-trimethylsilyloxyethyl)methylamine	76710-52-6	1314
Propyl trimethylsilyl propylphosphonate	111204-33-2	1314
1,2-Dimethylbutyl methyl ethylphosphonate-c		1315
1,4-Dimethylpentyl propylphosphonofluoride-a		1315
2,2-Dimethylcyclohexyl methylphosphonofluoride-a		1315
2-Methylcyclopentyl propylphosphonofluoride-a		1315
Dipropyl isopropylphosphonate	192698-90-1	1315
Pentyl ethylphosphonochloride		1316
Butyl <i>N,N</i> -dimethylphosphoramidocyanide	162085-87-2	1317
Propyl trimethylsilyl propylphosphonate		1317
Pinacolyl trimethylsilyl methylphosphonate	199116-10-4	1318
2,2-Dimethylcyclohexyl methylphosphonofluoride-b		1319
2-Methylcyclopentyl propylphosphonofluoride-b		1319
3,5-Dimethylcyclohexyl methylphosphonofluoride-br		1319
Ethyl 1-ethyl-2,2-dimethylpropyl methylphosphonate-a		1319
1-Isopropylbutyl propylphosphonofluoride-b		1321
1-Ethylpentyl isopropylphosphonofluoride-b		1322
Bis(<i>sec</i> -butyl) ethylphosphonate-a		1322
1-Ethyl-2-methylpentyl ethylphosphonofluoride-a		1323
1-Isobutyl-3-methylbutyl ethylphosphonofluoride		1323
1-Methylhexyl isopropylphosphonofluoride-a		1323
3,3-Dimethylbutyl methyl ethylphosphonate		1323
Methyl 1-methylpentyl ethylphosphonate		1323
Methyl pentyl ethylphosphonate	170275-31-7	1323
Methyl pinacolyl isopropylphosphonate-b		1324
<i>S</i> -Methyl <i>O</i> -propyl isopropylphosphonothiolate		1325
1-Ethylpentyl methyl methylphosphonate		1325
Bis(<i>sec</i> -butyl) ethylphosphonate-b		1325
3,5-Dimethylcyclohexyl methylphosphonofluoride-cr		1326
Methyl 1,3,3-trimethylbutyl ethylphosphonate-a		1326
Bis(<i>sec</i> -butyl) ethylphosphonate-c		1327
<i>tert</i> -Butyldimethylsilyl isopropyl methylphosphonate	126281-76-3	1327
1-Ethyl-2-methylbutyl propylphosphonofluoride-a		1328
2,3-Dimethylcyclohexyl methylphosphonofluoride-a		1328
3,5,5-Trimethylhexyl methylphosphonofluoride		1328
<i>tert</i> -Butyldimethylsilyl methyl ethylphosphonate		1328
1,4-Dimethylpentyl propylphosphonofluoride-b		1329
Ethyl 1-ethyl-2,2-dimethylpropyl methylphosphonate-b		1329
Ethyl 2-methoxyethyl isopropylphosphonate	192446-29-0	1329
1-Ethyl-2-methylpentyl ethylphosphonofluoride-b		1330
Methyl 1,3,3-trimethylbutyl ethylphosphonate-b		1330
Cycloheptyl methylphosphonofluoride	7284-83-5	1331
1,5-Dimethylhexyl ethylphosphonofluoride-a		1332
1-Ethyl-2-methylpentyl ethylphosphonofluoride-c		1332
3-Methylcyclohexyl ethylphosphonofluoride-a		1332
3-Methylcyclopentyl propylphosphonofluoride-a		1332
1-Methylhexyl isopropylphosphonofluoride-b		1333
2-Ethylcyclohexyl methylphosphonofluoride-a		1334
3-Methylcyclopentyl propylphosphonofluoride-b		1334
Ethyl 1-isopropyl-2-methylpropyl methylphosphonate		1334

(continued)

Compounds	CAS number	Retention index
1-Ethyl-2-methylbutyl propylphosphonofluoride-b		1335
1-Ethyl-2-methylpentyl ethylphosphonofluoride-d		1335
3-Methylcyclohexyl ethylphosphonofluoride-b		1335
1-Ethyl-2,2-dimethylpropyl methyl ethylphosphonate-a		1336
Methyl 1-methylhexyl methylphosphonate-a		1338
<i>O</i> -Propyl <i>O</i> -trimethylsilyl isopropylphosphonothionate		1339
1,2-Dimethylbutyl methyl isopropylphosphonate-a		1340
2,3-Dimethylcyclohexyl methylphosphonofluoride-b		1340
Pentyl trimethylsilyl methylphosphonate		1340
Methyl 1-methylhexyl methylphosphonate-b		1341
2-Ethylcyclohexyl methylphosphonofluoride-b		1343
Ethyl 1-isopropylbutyl methylphosphonate		1343
1,2-Dimethylbutyl methyl isopropylphosphonate-b		1344
1,5-Dimethylhexyl ethylphosphonofluoride-b		1344
1-Propylpentyl ethylphosphonofluoride-a		1344
Hexyl propylphosphonofluoride		1344
Methyl pinacolyl propylphosphonate-a		1344
1-Ethylpentyl propylphosphonofluoride-a		1345
4-Methylcyclohexyl ethylphosphonofluoride-a		1345
1,3-Dimethylbutyl methyl propylphosphonate-a		1346
1-Butylpentyl methylphosphonofluoride		1347
Bis(2,2-dimethylpropyl) methylphosphonate	53803-21-7	1347
1-Ethyl-2,2-dimethylpropyl methyl ethylphosphonate-b		1348
1-Propylpentyl ethylphosphonofluoride-b		1348
1,2-Dimethylbutyl methyl isopropylphosphonate-c		1349
2-Ethylcyclohexyl methylphosphonofluoride-c		1349
1,4-Dimethylpentyl ethyl methylphosphonate-a		1350
1,3-Dimethylbutyl methyl propylphosphonate-b		1351
Cyclohexyl isopropylphosphonofluoride	333416-55-0	1351
Methyl pinacolyl propylphosphonate-b		1351
1-Methylpentyl trimethylsilyl methylphosphonate		1352
Ethyl 2-methylcyclopentyl methylphosphonate		1352
2-Ethylbutyl methyl ethylphosphonate		1353
2-Ethylcyclohexyl methylphosphonofluoride-d		1353
Cyclopentyl ethylphosphonochloride		1353
Dipropyl propylphosphonate		1353
Methyl 2-methylpentyl ethylphosphonate		1353
1-Isopropyl-2-methylpropyl methyl ethylphosphonate		1354
Ethyl 2-diisopropylaminoethyl methylphosphonite	57856-11-8	1354
1-Methylhexyl propylphosphonofluoride-a		1355
2-Methylcyclohexyl ethylphosphonofluoride		1355
Cyclopentyl methyl ethylphosphonate		1355
1,2-Dimethylbutyl ethyl ethylphosphonate-a		1356
1-Ethylpentyl propylphosphonofluoride-b		1356
2,3-Dimethylcyclohexyl methylphosphonofluoride-c		1356
Methyl pentyl isopropylphosphonate		1356
1,4-Dimethylpentyl ethyl methylphosphonate-b		1357
3-Methylcyclohexyl ethylphosphonofluoride-c		1357
1,2-Dimethylbutyl ethyl ethylphosphonate-b		1358
Cyclopentylmethyl isopropylphosphonofluoride		1358
Methyl 3-methylbutyl propylphosphonate		1358
1-Cyclohexylethyl methylphosphonofluoride-a		1359

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Compounds	CAS number	Retention index
1-Ethylhexyl ethylphosphonofluoridate-a		1359
2,4,4-Trimethylpentyl isopropylphosphonofluoridate		1359
1,2-Dimethylbutyl ethyl ethylphosphonate-c		1361
Methyl 1,3,3-trimethylbutyl isopropylphosphonate-a		1361
Diisobutyl ethylphosphonate	7242-55-9	1362
1-Cyclohexylethyl methylphosphonofluoridate-b		1363
1-Isopropylbutyl methyl ethylphosphonate-a		1363
4-Methylcyclohexyl ethylphosphonofluoridate-b		1363
3,5-Dimethylcyclohexyl ethylphosphonofluoridate-a		1364
Bis(2-trimethylsilyloxyethyl)ethylamine		1364
Methyl 1,3,3-trimethylbutyl isopropylphosphonate-b		1364
1,2-Dimethylbutyl ethyl ethylphosphonate-d		1366
1-Ethylheptyl methylphosphonofluoridate-a		1366
Heptyl ethylphosphonofluoridate	162085-85-0	1366
1-Ethyl-2,2-dimethylpropyl methyl isopropylphosphonate-a		1367
1-Isobutyl-3-methylbutyl isopropylphosphonofluoridate		1367
4-Ethylcyclohexyl methylphosphonofluoridate-a		1367
Ethyl 3-methylcyclopentyl methylphosphonate		1367
1-Isopropylbutyl methyl ethylphosphonate-b		1368
2-Propylpentyl ethylphosphonofluoridate		1368
1-Ethylhexyl ethylphosphonofluoridate-b		1369
1-Methylhexyl propylphosphonofluoridate-b		1369
1-Ethyl-2-methylpropyl methyl propylphosphonate-a		1370
Methyl 3-methylpentyl ethylphosphonate		1370
Methyl 4-methylpentyl ethylphosphonate		1370
O-Propyl O-trimethylsilyl propylphosphonothionate		1371
1-Methylheptyl ethylphosphonofluoridate-a		1372
Cyclopentyl trimethylsilyl methylphosphonate	214042-75-8	1372
S-Methyl O-propyl propylphosphonothiolate		1373
1-Ethyl-2-methylpentyl isopropylphosphonofluoridate-a		1373
Ethyl N,N-diisopropylphosphoramidocyanidate		1373
O-Propyl S-trimethylsilyl propylphosphonothiolate		1374
1-Ethyl-2-methylpropyl methyl propylphosphonate-b		1374
1-Ethylheptyl methylphosphonofluoridate-b		1374
Cyclohexyl methyl methylphosphonate	7040-52-0	1374
Ethyl 1-ethylpentyl methylphosphonate-a		1374
Methyl 2-methylcyclopentyl ethylphosphonate		1375
1-Isobutyl-3-methylbutyl methylphosphonate		1376
Methyl 2,4,4-trimethylpentyl methylphosphonate		1376
Cyclopentylmethyl methyl methylphosphonate		1377
Ethyl 1,3,3-trimethylbutyl ethylphosphonate-a		1377
Ethyl 1-ethylpentyl methylphosphonate-b		1377
3-Methylcyclohexyl isopropylphosphonofluoridate-a		1378
Propyl N-methyl-N-propylphosphoramidocyanidate		1378
1,4-Dimethylpentyl methyl ethylphosphonate		1379
1-Ethyl-2-methylpentyl isopropylphosphonofluoridate-b		1379
1-Ethyl-2-methylpentyl methyl methylphosphonate-a		1379
1,5-Dimethylhexyl isopropylphosphonofluoridate-a		1380
1-Ethyl-2-methylpentyl isopropylphosphonofluoridate-c		1380
1-Methyloctyl methylphosphonofluoridate-a	211192-72-2	1380

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Compounds	CAS number	Retention index
<i>tert</i> -Butyldimethylsilyl ethyl ethylphosphonate		1381
2-Methylpentyl trimethylsilyl methylphosphonate		1382
3-Methylcyclohexyl isopropylphosphonofluoride-b		1382
Dipropyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidate		1382
Ethyl 1,3,3-trimethylbutyl ethylphosphonate-b		1382
1-Ethyl-2,2-dimethylpropyl methyl isopropylphosphonate-b		1383
1-Ethyl-2-methylpentyl methyl methylphosphonate-b		1383
1-Methylheptyl ethylphosphonofluoride-b		1383
1,2-Dimethylbutyl methyl propylphosphonate-a		1384
1-Ethyl-2-methylpentyl isopropylphosphonofluoride-d		1384
4-Ethylcyclohexyl methylphosphonofluoride-b		1384
1-Ethyl-2-methylpentyl methyl methylphosphonate-c		1385
1-Isopropyl-2-methylpropyl methyl isopropylphosphonate		1385
1,2-Dimethylbutyl methyl propylphosphonate-b		1387
Butyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanide		1387
Ethyl 1-methylhexyl methylphosphonate-a		1387
1,2-Dimethylbutyl ethyl isopropylphosphonate-a		1388
1-Ethylbutyl methyl propylphosphonate-a		1388
2-Ethylhexyl ethylphosphonofluoride		1388
Cyclopentyl methyl isopropylphosphonate		1388
1,2-Dimethylbutyl methyl propylphosphonate-c		1389
Ethyl 1-ethyl-2,2-dimethylpropyl ethylphosphonate-a		1389
1,2-Dimethylbutyl ethyl isopropylphosphonate-b		1390
1-Methyloctyl methylphosphonofluoride-b	211192-72-2	1390
1-Propylpentyl isopropylphosphonofluoride-a		1390
Methyl 3-methylcyclopentyl ethylphosphonate-a		1390
<i>tert</i> -Butyldimethylsilyl propyl methylphosphonate		1390
1,5-Dimethylhexyl isopropylphosphonofluoride-b		1391
1-Ethylbutyl methyl propylphosphonate-b		1391
1-Isopropylbutyl methyl isopropylphosphonate-a		1391
2,4,4-Trimethylpentyl propylphosphonofluoride		1391
4-Methylcyclohexyl isopropylphosphonofluoride-a		1391
Cyclohexyl propylphosphonofluoride	28364-21-8	1391
Methyl 3-methylcyclopentyl ethylphosphonate-b		1392
Cyclopentylmethyl propylphosphonofluoride		1393
1,2-Dimethylbutyl ethyl isopropylphosphonate-c		1394
1-Propylpentyl isopropylphosphonofluoride-b		1394
Methyl 3-methylcyclohexyl methylphosphonate-a		1394
Bis(1-methylbutyl) methylphosphonate-a		1395
Ethyl 1-methylhexyl methylphosphonate-b		1395
1,5-Dimethylhexyl methyl methylphosphonate-a		1396
1,2-Dimethylbutyl ethyl isopropylphosphonate-d		1399
1,5-Dimethylhexyl methyl methylphosphonate-b		1399
1-Ethylpentyl methyl ethylphosphonate-a		1399
3-Methylpentyl trimethylsilyl methylphosphonate		1399
Methyl 1-methylpentyl propylphosphonate-a		1399
1-Ethyl-2-methylpentyl propylphosphonofluoride-a		1400
1-Isopropylbutyl methyl isopropylphosphonate-b		1400
Methyl 1-propylpentyl methylphosphonate		1400
3,5-Dimethylcyclohexyl ethylphosphonofluoride-b		1401
Methyl 1-methylpentyl propylphosphonate-b		1401
1-Ethylpentyl methyl ethylphosphonate-b		1402

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Compounds	CAS number	Retention index
Ethyl 1-ethyl-2,2-dimethylpropyl ethylphosphonate-b		1402
Methyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	108490-92-2	1402
3,3-Dimethylbutyl methyl propylphosphonate		1403
Methyl pentyl propylphosphonate		1403
<i>tert</i> -Butyldimethylsilyl methyl propylphosphonate		1403
Bis(1-methylbutyl) methylphosphonate-b		1404
Methyl 1,3,3-trimethylbutyl propylphosphonate		1404
3-Methylcyclohexyl isopropylphosphonofluoride-c		1405
1-Isobutyl-3-methylbutyl propylphosphonofluoride		1406
Ethyl 1-isopropyl-2-methylpropyl ethylphosphonate		1406
Methyl <i>N,N</i> -dipropylphosphoramidocyanide		1406
1-Ethyl-2-methylpentyl propylphosphonofluoride-b		1407
3,5-Dimethylcyclohexyl isopropylphosphonofluoride-a		1407
3-Methylcyclohexyl isopropylphosphonofluoride-d		1407
Methyl 4-methylcyclohexyl methylphosphonate-a		1407
<i>tert</i> -Butyldimethylsilyl isopropyl ethylphosphonate		1407
2-Methylcyclohexyl isopropylphosphonofluoride-a		1408
1,5-Dimethylhexyl propylphosphonofluoride-a		1409
3,5-Dimethylcyclohexyl ethylphosphonofluoride-c		1409
Heptyl isopropylphosphonofluoride		1409
Methyl 2-methylcyclohexyl methylphosphonate-a		1409
2-Methylcyclohexyl isopropylphosphonofluoride-b		1410
2,6-Dimethylcyclohexyl ethylphosphonofluoride		1411
Ethyl 1,3,3-trimethylbutyl isopropylphosphonate-a		1411
Tris(2-chloroethyl)amine	555-77-1	1411
1,4-Dimethylpentyl methyl isopropylphosphonate		1412
1-Ethyl-2,2-dimethylpropyl methyl propylphosphonate-a		1412
1-Ethyl-2-methylpentyl propylphosphonofluoride-c		1412
1-Ethylhexyl isopropylphosphonofluoride-a		1412
2-Cyclohexylethyl methylphosphonofluoride		1412
Ethyl 1-isopropylbutyl ethylphosphonate-a		1412
Pentyl <i>N,N</i> -dimethylphosphoramidocyanide	148461-87-4	1412
Bis(1-methylbutyl) methylphosphonate-c		1413
Hexyl methyl ethylphosphonate	98958-69-1	1413
2-Propylpentyl isopropylphosphonofluoride		1414
3-Cyclopentylpropyl methylphosphonofluoride		1414
4-Methylcyclohexyl isopropylphosphonofluoride-b		1414
Cyclohexylmethyl ethylphosphonofluoride		1414
Methyl 2-methylcyclohexyl methylphosphonate-b		1414
<i>sec</i> -Butyl <i>tert</i> -butyldimethylsilyl methylphosphonate		1414
1-Ethyl-2-methylpentyl propylphosphonofluoride-d		1415
Ethyl 1,3,3-trimethylbutyl isopropylphosphonate-b		1415
Ethyl 1-isopropylbutyl ethylphosphonate-b		1416
Bis(2-methoxyethyl) methylphosphonate	6069-09-6	1418
Ethyl 1-ethyl-2,2-dimethylpropyl isopropylphosphonate-a		1418
Methyl 1-methylhexyl ethylphosphonate		1418
1-Ethylhexyl methyl methylphosphonate		1419
1-Methylheptyl isopropylphosphonofluoride-a		1419
3,5,5-Trimethylhexyl ethylphosphonofluoride		1419
Methyl 2-methylcyclopentyl isopropylphosphonate		1420
Methyl 3-methylcyclohexyl methylphosphonate-b		1420

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Compounds	CAS number	Retention index
Bis(2,2-dimethylpropyl) ethylphosphonate		1422
Dipropyl <i>N</i> -isopropyl- <i>N</i> -methylphosphoramidate		1422
1-Ethyl-2,2-dimethylpropyl methyl propylphosphonate-b		1423
1-Propylpentyl propylphosphonofluoridate-a		1423
2-Ethylcyclohexyl ethylphosphonofluoridate-a		1423
1-Ethylhexyl isopropylphosphonofluoridate-b		1424
1,4-Dimethylpentyl ethyl ethylphosphonate-a		1425
3,5-Dimethylcyclohexyl methyl methylphosphonate-a		1425
Ethyl 1-isobutyl-3-methylbutyl methylphosphonate		1425
1,5-Dimethylhexyl propylphosphonofluoridate-b		1426
2,3-Dimethylcyclohexyl ethylphosphonofluoridate-a		1426
Bis(2-trimethylsilyloxyethyl)sulfide	20486-03-7	1426
1-Isopropyl-2-methylpropyl methyl propylphosphonate		1428
1-Propylpentyl propylphosphonofluoridate-b		1428
Ethyl 1-ethyl-2-methylpentyl methylphosphonate-a		1428
Ethyl 2-methylcyclopentyl ethylphosphonate		1428
Heptyl methyl methylphosphonate	170275-60-2	1428
1,4-Dimethylpentyl ethyl ethylphosphonate-b		1429
Methyl 4-methylcyclohexyl methylphosphonate-b		1429
1,2-Dimethylbutyl ethyl propylphosphonate-a		1430
Bis(1-ethylpropyl) methylphosphonate	5828-67-1	1430
Ethyl 2,4,4-trimethylpentyl methylphosphonate		1430
Methyl 3-methylcyclopentyl isopropylphosphonate-a		1430
<i>tert</i> -Butyldimethylsilyl isobutyl methylphosphonate		1430
1-Ethylpentyl methyl isopropylphosphonate-a		1431
1-Methylheptyl isopropylphosphonofluoridate-b		1431
2,3-Dimethylcyclohexyl ethylphosphonofluoridate-b		1431
2-Ethylbutyl methyl propylphosphonate		1431
2-Ethylcyclohexyl ethylphosphonofluoridate-b		1431
Ethyl 1-ethyl-2-methylpentyl methylphosphonate-b		1431
Cycloheptyl ethylphosphonofluoridate		1432
Methyl 2-methylpentyl propylphosphonate		1432
1,2-Dimethylbutyl ethyl propylphosphonate-b		1433
2,3-Dimethylcyclohexyl ethylphosphonofluoridate-c		1433
Methyl 2-propylpentyl methylphosphonate		1433
1-Butylpentyl ethylphosphonofluoridate		1434
1-Isopropylbutyl methyl propylphosphonate-a		1434
1-Ethylpentyl methyl isopropylphosphonate-b		1435
2-Ethylhexyl isopropylphosphonofluoridate		1435
Cyclopentylmethyl ethyl methylphosphonate		1435
Hexyl trimethylsilyl methylphosphonate		1435
Ethyl 1-ethyl-2-methylpentyl methylphosphonate-c		1436
Methyl 1-methylheptyl methylphosphonate-a		1436
Methyl 3-methylcyclopentyl isopropylphosphonate-b		1436
Ethyl 1-ethyl-2,2-dimethylpropyl isopropylphosphonate-b		1437
1,2-Dimethylbutyl ethyl propylphosphonate-c		1438
2-Ethylcyclohexyl ethylphosphonofluoridate-c		1438
Cyclopentyl methyl propylphosphonate		1438
Ethyl 1-isopropyl-2-methylpropyl isopropylphosphonate		1438
Methyl 1-methylheptyl methylphosphonate-b		1439
Ethyl 1-isopropylbutyl isopropylphosphonate-a		1440

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Compounds	CAS number	Retention index
1-Isopropylbutyl methyl propylphosphonate-b		1441
2-Ethylcyclohexyl ethylphosphonofluoridate-d		1441
3-Methylcyclohexyl propylphosphonofluoridate-a		1442
Ethyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	20820-80-8	1442
1-Ethylhexyl propylphosphonofluoridate-a		1443
Ethyl phenyl methylphosphonate		1443
3-Methylcyclohexyl propylphosphonofluoridate-b		1444
1-Isobutyl-3-methylbutyl methyl ethylphosphonate		1445
Heptyl propylphosphonofluoridate		1445
1,5-Dimethylhexyl ethyl methylphosphonate-a		1446
2-Ethylhexyl methyl methylphosphonate		1446
2-Methylcyclohexyl propylphosphonofluoridate		1446
Dibutyl ethylphosphonate	2404-58-2	1446
Ethyl 3-methylcyclopentyl ethylphosphonate		1446
2-Propylpentyl propylphosphonofluoridate		1447
3,5-Dimethylcyclohexyl propylphosphonofluoridate-a		1447
Ethyl 1-isopropylbutyl isopropylphosphonate-b		1447
Ethyl 3-methylcyclohexyl methylphosphonate-a		1447
<i>tert</i> -Butyldimethylsilyl 2,2-dimethylpropyl methylphosphonate		1447
1-Methylheptyl propylphosphonofluoridate-a		1449
Ethyl 1-ethylpentyl ethylphosphonate		1449
4-Methylcyclohexyl propylphosphonofluoridate		1450
Ethyl 1-propylpentyl methylphosphonate		1450
Ethyl 3-methylcyclohexyl methylphosphonate-b		1450
Methyl 3-methylpentyl propylphosphonate		1450
Methyl 4-methylpentyl propylphosphonate		1450
1-Ethyl-2-methylpentyl methyl ethylphosphonate-a		1451
Cyclooctyl methylphosphonofluoridate	14719-38-1	1451
Methyl 1-methylhexyl isopropylphosphonate		1451
1-Cyclohexylethyl ethylphosphonofluoridate-a		1452
2,3-Dimethylcyclohexyl ethylphosphonofluoridate-d		1452
3,5-Dimethylcyclohexyl isopropylphosphonofluoridate-b		1452
Hexyl methyl isopropylphosphonate		1452
1,4-Dimethylpentyl methyl propylphosphonate-a		1453
1,5-Dimethylhexyl ethyl methylphosphonate-b		1453
1-Ethyl-2-methylpentyl methyl ethylphosphonate-b		1453
Cyclohexyl methyl ethylphosphonate	170275-49-7	1453
Ethyl 1,3,3-trimethylbutyl propylphosphonate		1453
1-Ethylheptyl ethylphosphonofluoridate-a		1454
4-Ethylcyclohexyl ethylphosphonofluoridate-a		1454
1,4-Dimethylpentyl ethyl isopropylphosphonate-a		1456
1,4-Dimethylpentyl methyl propylphosphonate-b		1456
1-Ethylhexyl propylphosphonofluoridate-b		1456
Methyl 2-methylcyclopentyl propylphosphonate-a		1456
1-Cyclohexylethyl ethylphosphonofluoridate-b		1458
1-Ethyl-2-methylpentyl methyl ethylphosphonate-c		1458
Isopropyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	21068-52-0	1458
Methyl 2-methylcyclopentyl propylphosphonate-b		1458
2,6-Dimethylcyclohexyl methyl methylphosphonate-a		1459
3,5-Dimethylcyclohexyl isopropylphosphonofluoridate-c		1459

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Compounds	CAS number	Retention index
1,4-Dimethylpentyl ethyl isopropylphosphonate-b		1460
Cyclopentylmethyl methyl ethylphosphonate		1460
Ethyl 2-methylcyclopentyl isopropylphosphonate		1460
Ethyl 1-ethyl-2,2-dimethylpropyl propylphosphonate-a		1461
Ethyl 4-methylcyclohexyl methylphosphonate-a		1461
Propyl <i>N,N</i> -diisopropylphosphoramidocyanide		1461
3,5,5-Trimethylhexyl isopropylphosphonofluoride		1462
Ethyl <i>N,N</i> -dipropylphosphoramidocyanide		1462
Octyl ethylphosphonofluoride		1462
Nonyl methylphosphonofluoride	211192-74-4	1463
Cyclohexylmethyl isopropylphosphonofluoride		1464
Dipropyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidate		1464
Ethyl 1-methylhexyl ethylphosphonate-a		1464
Ethyl 2-methylcyclohexyl methylphosphonate-a	161585-25-7	1464
2-Isopropyl-5-methylcyclohexyl methylphosphonofluoride-a		1465
Tris(2-chlorovinyl)arsine	40334-70-1	1465
1-Ethylheptyl ethylphosphonofluoride-b		1466
1-Methylheptyl propylphosphonofluoride-b		1466
3,5-Dimethylcyclohexyl methyl methylphosphonate-b		1466
3,7-Dimethyloctyl methylphosphonofluoride		1466
2,6-Dimethylcyclohexyl isopropylphosphonofluoride-a		1467
2,6-Dimethylcyclohexyl methyl methylphosphonate-b		1467
2-Ethylhexyl propylphosphonofluoride		1467
Ethyl 1-ethylhexyl methylphosphonate-a		1467
Bis(1-methylbutyl) ethylphosphonate-a		1468
Ethyl 2-methylcyclohexyl methylphosphonate-b	161585-25-7	1468
1-Methyloctyl ethylphosphonofluoride-a		1469
3,5-Dimethylcyclohexyl methyl methylphosphonate-c		1469
Ethyl 1-methylhexyl ethylphosphonate-b		1469
2,6-Dimethylcyclohexyl isopropylphosphonofluoride-b		1470
Butyl <i>tert</i> -butyldimethylsilyl methylphosphonate		1470
2- <i>tert</i> -Butylcyclohexyl methylphosphonofluoride-a		1471
Butyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanide		1471
Ethyl 1-ethylhexyl methylphosphonate-b		1471
Methyl 3-methylcyclopentyl propylphosphonate-a		1471
1-Ethylpentyl methyl propylphosphonate-a		1472
1-Isobutyl-3-methylbutyl methyl isopropylphosphonate		1472
2-Isopropyl-5-methylcyclohexyl methylphosphonofluoride-b		1472
Ethyl 3-methylcyclohexyl methylphosphonate-c		1472
Ethyl 3-methylcyclopentyl isopropylphosphonate-a		1472
Methyl 1-propylpentyl ethylphosphonate		1473
Ethyl 1-ethyl-2,2-dimethylpropyl propylphosphonate-b		1474
Methyl 3-methylcyclopentyl propylphosphonate-b		1474
<i>tert</i> -Butyldimethylsilyl isopropyl propylphosphonate		1474
1,5-Dimethylhexyl methyl ethylphosphonate		1475
Methyl 3-methylcyclohexyl ethylphosphonate-a		1475
2-Ethylcyclohexyl isopropylphosphonofluoride-a		1476
3,5-Dimethylcyclohexyl ethyl methylphosphonate-a		1476

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Compounds	CAS number	Retention index
Ethyl 1-isopropyl-2-methylpropyl propylphosphonate		1476
Ethyl 3-methylcyclohexyl methylphosphonate-d		1476
Methyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1476
Bis(1-methylbutyl) ethylphosphonate-b		1477
Ethyl 3-methylcyclopentyl isopropylphosphonate-b		1477
1-Butylpentyl isopropylphosphonofluoridate		1478
1-Ethylpentyl methyl propylphosphonate-b		1478
4-Ethylcyclohexyl ethylphosphonofluoridate-b		1478
Bis(3-methylbutyl) methylphosphonate	2452-70-2	1478
<i>tert</i> -Butyldimethylsilyl 1-methylbutyl methylphosphonate		1478
2,3-Dimethylcyclohexyl isopropylphosphonofluoridate-a		1479
Ethyl 1-ethylpentyl isopropylphosphonate-a		1479
1-Ethyl-2-methylpentyl methyl isopropylphosphonate-a		1480
Cycloheptyl isopropylphosphonofluoridate		1480
Ethyl 1-isopropylbutyl propylphosphonate-a		1480
1-Methyloctyl ethylphosphonofluoridate-b		1481
2- <i>tert</i> -Butylcyclohexyl methylphosphonofluoridate-b		1481
Ethyl 1-ethylpentyl isopropylphosphonate-b		1481
Ethyl heptyl methylphosphonate		1481
2,3-Dimethylcyclohexyl isopropylphosphonofluoridate-b		1482
Bis(2-methylbutyl) methylphosphonate		1482
Cyclohexylmethyl methyl methylphosphonate		1482
Methyl 3,5,5-trimethylhexyl methylphosphonate		1482
1-Methylnonyl methylphosphonofluoridate-a	211192-71-1	1483
2-Ethylcyclohexyl isopropylphosphonofluoridate-b		1483
2-Ethylcyclohexyl methyl methylphosphonate-a		1483
Ethyl 1-methylheptyl methylphosphonate-a	22583-45-5	1483
<i>tert</i> -Butyldimethylsilyl 1,2-dimethylpropyl methylphosphonate		1483
1-Ethyl-2-methylpentyl methyl isopropylphosphonate-b		1484
Bis(1-methylbutyl) ethylphosphonate-c		1484
Ethyl 2-propylpentyl methylphosphonate		1484
Ethyl 4-methylcyclohexyl methylphosphonate-b		1484
Pentyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanidate		1484
2,3-Dimethylcyclohexyl methyl methylphosphonate-a		1485
Cyclohexyl trimethylsilyl methylphosphonate	199116-11-5	1486
Ethyl 1-isopropylbutyl propylphosphonate-b		1486
1-Ethyl-2-methylpentyl methyl isopropylphosphonate-c		1487
Methyl 2,4,4-trimethylpentyl isopropylphosphonate		1487
Methyl 4-methylcyclohexyl ethylphosphonate-a		1487
<i>tert</i> -Butyldimethylsilyl 1-ethylpropyl methylphosphonate		1487
1-Butylpentyl methyl methylphosphonate		1488
2,3-Dimethylcyclohexyl methyl methylphosphonate-b		1489
2-Ethylcyclohexyl isopropylphosphonofluoridate-c		1490
3,5-Dimethylcyclohexyl propylphosphonofluoridate-b		1490
Ethyl 1-isobutyl-3-methylbutyl ethylphosphonate		1491
Methyl 1-methylhexyl propylphosphonate-a		1491

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Compounds	CAS number	Retention index
1-Ethylhexyl methyl ethylphosphonate-a		1492
2-Ethylcyclohexyl isopropylphosphonofluoride-d		1492
Cycloheptyl methyl methylphosphonate		1492
Ethyl 1-methylheptyl methylphosphonate-b	22583-45-5	1492
2,3-Dimethylcyclohexyl methyl methylphosphonate-c		1493
1-Methylnonyl methylphosphonofluoride-b	211192-71-1	1494
Bis(1-ethylpropyl) ethylphosphonate		1494
Hexyl methyl propylphosphonate		1494
1-Ethylhexyl methyl ethylphosphonate-b		1495
3,5,5-Trimethylhexyl propylphosphonofluoride		1495
3,5-Dimethylcyclohexyl propylphosphonofluoride-c		1495
Methyl 1-methylhexyl propylphosphonate-b		1495
Cyclopentylmethyl methyl isopropylphosphonate		1496
Ethyl 1-methylhexyl isopropylphosphonate-a		1496
2,6-Dimethylcyclohexyl propylphosphonofluoride		1497
2-Ethylcyclohexyl methyl methylphosphonate-b		1497
Ethyl 2-ethylhexyl methylphosphonate	88795-46-4	1497
2-tert-Butylcyclohexyl methylphosphonofluoride-c		1498
3,5-Dimethylcyclohexyl methyl ethylphosphonate-a		1499
Methyl 2-methylcyclohexyl ethylphosphonate-a		1499
1,4-Dimethylpentyl ethyl propylphosphonate		1500
1-Ethylheptyl isopropylphosphonofluoride-a		1500
Cyclohexyl methyl isopropylphosphonate		1500
Cyclohexylmethyl propylphosphonofluoride		1500
Ethyl 1-ethyl-2-methylpentyl ethylphosphonate-a		1500
Ethyl 1-methylhexyl isopropylphosphonate-b		1500
Methyl 1-propylpentyl isopropylphosphonate		1501
tert-Butyldimethylsilyl 2-methoxyethyl methylphosphonate		1501
1-Cyclohexylethyl isopropylphosphonofluoride-a		1502
2,3-Dimethylcyclohexyl isopropylphosphonofluoride-c		1502
4-Ethylcyclohexyl isopropylphosphonofluoride-a		1502
2-Ethylcyclohexyl methyl methylphosphonate-c		1503
Ethyl 1-ethyl-2-methylpentyl ethylphosphonate-b		1504
Ethyl 2,4,4-trimethylpentyl ethylphosphonate		1504
Ethyl 2-methylcyclopentyl propylphosphonate-a		1504
Methyl 3-methylcyclohexyl ethylphosphonate-b		1504
2-tert-Butylcyclohexyl methylphosphonofluoride-d		1505
Methyl 2-methylcyclohexyl ethylphosphonate-b		1505
1,5-Dimethylhexyl methyl isopropylphosphonate		1506
2-Ethylcyclohexyl propylphosphonofluoride-a		1506
Ethyl 1-ethyl-2-methylpentyl ethylphosphonate-c		1506
2,3-Dimethylcyclohexyl methyl methylphosphonate-d		1507
Dipinacolyl methylphosphonate	7040-58-6	1507
Ethyl 2-methylcyclopentyl propylphosphonate-b		1507
Methyl 3-methylcyclohexyl isopropylphosphonate-a		1507
1-Cyclohexylethyl isopropylphosphonofluoride-b		1508
2-Cyclohexylethyl ethylphosphonofluoride		1508
Methyl 2-propylpentyl ethylphosphonate		1508
3-Cyclopentylpropyl ethylphosphonofluoride		1509
tert-Butyldimethylsilyl 3-methylbutyl methylphosphonate		1509
4-tert-Butylcyclohexyl methylphosphonofluoride-a		1510
Octyl isopropylphosphonofluoride		1510

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Compounds	CAS number	Retention index
2,3-Dimethylcyclohexyl methyl methylphosphonate-e		1511
1-Butylpentyl propylphosphonofluoridate		1512
1-Cyclohexylethyl methyl methylphosphonate-a		1512
Cyclopentylmethyl ethyl ethylphosphonate		1513
Heptyl methyl ethylphosphonate	169662-35-5	1513
Hexyl <i>N,N</i> -dimethylphosphoramidocyanidate	162085-90-7	1513
Methyl 4-methylcyclohexyl ethylphosphonate-b		1513
<i>tert</i> -Butyldimethylsilyl 2-methylbutyl methylphosphonate		1513
1-Ethylheptyl isopropylphosphonofluoridate-b		1514
Methyl 1-methylheptyl ethylphosphonate		1514
1-Isobutyl-3-methylbutyl methyl propylphosphonate		1515
1-Methyloctyl isopropylphosphonofluoridate-a		1515
2,6-Dimethylcyclohexyl ethyl methylphosphonate-a		1515
1-Ethylheptyl methyl methylphosphonate		1516
2,3-Dimethylcyclohexyl propylphosphonofluoridate-a		1516
2-Ethylcyclohexyl propylphosphonofluoridate-b		1516
3,5-Dimethylcyclohexyl ethyl methylphosphonate-b		1518
4-Ethylcyclohexyl methyl methylphosphonate-a		1518
Ethyl 1-ethylpentyl propylphosphonate-a		1518
Ethyl 1-isobutyl-3-methylbutyl isopropylphosphonate		1518
Ethyl 1-propylpentyl ethylphosphonate		1518
1,5-Dimethylhexyl ethyl ethylphosphonate-a		1519
Cycloheptyl propylphosphonofluoridate	345239-11-4	1520
Ethyl 3-methylcyclopentyl propylphosphonate-a		1520
Methyl 4-methylcyclohexyl isopropylphosphonate-a		1520
1-Cyclohexylethyl methyl methylphosphonate-b		1521
2,3-Dimethylcyclohexyl propylphosphonofluoridate-b		1521
Ethyl 1-ethylpentyl propylphosphonate-b		1521
<i>O</i> -Ethyl <i>O</i> -2-ethylthioethyl methylphosphonothionate	89980-25-6	1522
1-Ethyl-2-methylpentyl methyl propylphosphonate-a		1522
1-Ethylhexyl methyl isopropylphosphonate-a		1522
Ethyl 3-methylcyclopentyl propylphosphonate-b		1522
1,5-Dimethylhexyl ethyl ethylphosphonate-b		1523
2-Ethylcyclohexyl propylphosphonofluoridate-c		1523
3,5-Dimethylcyclohexyl ethyl methylphosphonate-c		1523
Ethyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate	98543-25-0	1523
Ethyl 3-methylcyclohexyl ethylphosphonate-a		1524
2,6-Dimethylcyclohexyl ethyl methylphosphonate-b		1525
3-Cyclohexylpropyl methylphosphonofluoridate	211192-73-3	1525
1-Ethyl-2-methylpentyl methyl propylphosphonate-b		1527
1-Ethylhexyl methyl isopropylphosphonate-b		1527
Methyl octyl methylphosphonate	73060-40-9	1527
3,5-Dimethylcyclohexyl methyl isopropylphosphonate-a		1528
Ethyl 1-ethyl-2-methylpentyl isopropylphosphonate-a		1528
1-Methyloctyl isopropylphosphonofluoridate-b		1529
2-Ethylcyclohexyl propylphosphonofluoridate-d		1529
2-Ethylhexyl methyl ethylphosphonate		1530
4-Ethylcyclohexyl isopropylphosphonofluoridate-b		1530
1-Ethyl-2-methylpentyl methyl propylphosphonate-c		1531
Ethyl 1-ethyl-2-methylpentyl isopropylphosphonate-b		1531
Methyl 2,4,4-trimethylpentyl propylphosphonate		1531
1-Ethylheptyl propylphosphonofluoridate-a		1532

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Compounds	CAS number	Retention index
Isopropyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate	162085-92-9	1533
1-Butylpentyl ethyl methylphosphonate		1534
Ethyl 3,5,5-trimethylhexyl methylphosphonate		1534
1-Cyclohexylethyl propylphosphonofluoridate-a		1535
Ethyl 1-ethyl-2-methylpentyl isopropylphosphonate-c		1535
Ethyl 2,4,4-trimethylpentyl isopropylphosphonate		1535
Ethyl 2-ethylcyclohexyl methylphosphonate-a		1535
Methyl 1-methyloctyl methylphosphonate-a		1535
Methyl 2-methylcyclohexyl isopropylphosphonate-a		1535
Cyclohexyl methyl propylphosphonate		1536
Propyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	56217-62-0	1536
2,3-Dimethylcyclohexyl propylphosphonofluoridate-c		1538
Ethyl 4-methylcyclohexyl ethylphosphonate-a		1538
Methyl 1-methyloctyl methylphosphonate-b		1538
Methyl 3-methylcyclohexyl isopropylphosphonate-b		1538
Bis(1,3-dimethylbutyl) ethylphosphonate-a		1539
Ethyl 1-methylhexyl propylphosphonate		1539
Methyl 2-propylpentyl isopropylphosphonate		1539
2,3-Dimethylcyclohexyl ethyl methylphosphonate-a		1540
Cyclohexylmethyl ethyl methylphosphonate		1540
Ethyl 1-ethylhexyl ethylphosphonate		1540
2,3-Dimethylcyclohexyl propylphosphonofluoridate-d		1541
4-Ethylcyclohexyl propylphosphonofluoridate-a		1541
2,6-Dimethylcyclohexyl methyl ethylphosphonate-a		1542
Cyclopentylmethyl methyl propylphosphonate		1542
1-Propylheptyl ethylphosphonofluoridate-a		1543
Ethyl 2-methylcyclohexyl ethylphosphonate-a		1543
Methyl 1-propylpentyl propylphosphonate-a		1543
Octyl propylphosphonofluoridate		1543
2,6-Dimethylcyclohexyl methyl ethylphosphonate-b		1544
1-Cyclohexylethyl propylphosphonofluoridate-b		1545
2,3-Dimethylcyclohexyl ethyl methylphosphonate-b		1545
3,5-Dimethylcyclohexyl methyl ethylphosphonate-b		1545
4-Ethylcyclohexyl methyl methylphosphonate-b		1545
Methyl 1-propylpentyl propylphosphonate-b		1545
Methyl 2-methylcyclohexyl isopropylphosphonate-b		1545
1,5-Dimethylhexyl methyl propylphosphonate-a		1546
Cycloheptyl ethyl methylphosphonate	22583-41-1	1546
Ethyl 1-propylpentyl isopropylphosphonate		1546
3,5-Dimethylcyclohexyl ethyl ethylphosphonate-a		1547
Bis(1,3-dimethylbutyl) ethylphosphonate-b		1547
Cyclopentylmethyl ethyl isopropylphosphonate		1547
Heptyl methyl isopropylphosphonate		1547
Methyl 1-methylheptyl isopropylphosphonate		1547
1-Ethylheptyl propylphosphonofluoridate-b		1548
1-Methyloctyl propylphosphonofluoridate-a		1548
Ethyl 2-ethylcyclohexyl methylphosphonate-b		1548
Ethyl 2-methylcyclohexyl ethylphosphonate-b		1548
3,5-Dimethylcyclohexyl methyl ethylphosphonate-c		1549
Butyl <i>N,N</i> -diisopropylphosphoramidocyanidate		1549
Methyl 4-methylcyclohexyl isopropylphosphonate-b		1549
Bis(3-methylbutyl) ethylphosphonate		1550
Cyclooctyl ethylphosphonofluoridate		1550

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Compounds	CAS number	Retention index
Methyl 3-methylcyclohexyl propylphosphonate-a		1550
1,5-Dimethylhexyl methyl propylphosphonate-b		1551
1-Propylheptyl ethylphosphonofluoride-b		1551
Ethyl 3-methylcyclohexyl ethylphosphonate-b		1551
1,5-Dimethylhexyl ethyl isopropylphosphonate-a		1552
<i>sec</i> -Butyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		1552
4- <i>tert</i> -Butylcyclohexyl methylphosphonofluoride-b		1553
Bis(2-methylbutyl) ethylphosphonate		1553
Ethyl 3-methylcyclohexyl isopropylphosphonate-a		1553
<i>tert</i> -Butyldimethylsilyl pentyl methylphosphonate		1553
1,5-Dimethylhexyl ethyl isopropylphosphonate-b		1554
Ethyl 3-methylcyclohexyl ethylphosphonate-c		1554
Methyl 3-methylcyclohexyl propylphosphonate-b		1554
Propyl <i>N,N</i> -dipropylphosphoramidocyanide		1554
<i>tert</i> -Butyldimethylsilyl pinacolyl methylphosphonate	126281-77-4	1554
2-Cyclohexylethyl isopropylphosphonofluoride		1555
Bis(1,3-dimethylbutyl) ethylphosphonate-c		1556
Ethyl 2-ethylcyclohexyl methylphosphonate-c		1556
Ethyl 2-propylpentyl ethylphosphonate		1556
Nonyl ethylphosphonofluoride	171741-07-4	1556
1-Butylpentyl methyl ethylphosphonate		1557
2,3-Dimethylcyclohexyl ethyl methylphosphonate-c		1557
Ethyl heptyl ethylphosphonate		1557
<i>O</i> -Ethyl <i>S</i> -3-dimethylaminopropyl methylphosphonothiolate		1558
Ethyl 1-isobutyl-3-methylbutyl propylphosphonate		1558
Methyl 3,5,5-trimethylhexyl ethylphosphonate		1558
<i>sec</i> -Butyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1558
3,7-Dimethyloctyl ethylphosphonofluoride		1559
Ethyl 1-methylheptyl ethylphosphonate-a		1559
1-Cyclohexylethyl ethyl methylphosphonate-a		1561
Ethyl 1-ethylheptyl methylphosphonate-a		1561
2,3-Dimethylcyclohexyl ethyl methylphosphonate-d		1562
2-Ethylcyclohexyl methyl ethylphosphonate-a		1562
2-Ethylhexyl trimethylsilyl methylphosphonate		1562
1-Ethylhexyl methyl propylphosphonate-a		1563
2-Ethylhexyl methyl isopropylphosphonate		1563
3-Cyclopentylpropyl isopropylphosphonofluoride		1563
Ethyl 1-methylheptyl ethylphosphonate-b		1563
Decyl methylphosphonofluoride	193090-25-4	1564
Ethyl 4-methylcyclohexyl ethylphosphonate-b		1564
Cyclohexylmethyl methyl ethylphosphonate		1565
Methyl 4-methylcyclohexyl propylphosphonate-a		1565
1-Methyloctyl propylphosphonofluoride-b		1566
Ethyl 1-ethylheptyl methylphosphonate-b		1566
2,3-Dimethylcyclohexyl methyl ethylphosphonate-a		1567
Ethyl 1-ethyl-2-methylpentyl propylphosphonate-a		1567
1-Methylnonyl ethylphosphonofluoride-a		1568
2- <i>tert</i> -Butylcyclohexyl ethylphosphonofluoride-a		1568
Dipentyl methylphosphonate	1000-36-8	1568
Ethyl 4-methylcyclohexyl isopropylphosphonate-a		1568
Pentyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanide		1568

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Compounds	CAS number	Retention index
<i>tert</i> -Butyldimethylsilyl 1-methylpentyl methylphosphonate		1568
2,6-Dimethylcyclohexyl methyl isopropylphosphonate-a		1569
3,3,5,5-Tetramethylcyclohexyl propylphosphonofluoridate	333416-72-1	1569
4-Ethylcyclohexyl propylphosphonofluoridate-b		1569
Bis(2-chloroethylthio)methane	63869-13-6	1569
Ethyl 1-ethylhexyl isopropylphosphonate-a		1569
Ethyl 2-ethylhexyl ethylphosphonate		1569
1-Ethylhexyl methyl propylphosphonate-b		1570
2,3-Dimethylcyclohexyl methyl ethylphosphonate-b		1570
Ethyl 1-ethyl-2-methylpentyl propylphosphonate-b		1570
Ethyl 4-ethylcyclohexyl methylphosphonate-a		1570
Ethyl 1-ethylhexyl isopropylphosphonate-b		1572
3,5-Dimethylcyclohexyl ethyl isopropylphosphonate-a		1573
2,3-Dimethylcyclohexyl methyl ethylphosphonate-c		1574
Ethyl 1-ethyl-2-methylpentyl propylphosphonate-c		1574
2-Ethylcyclohexyl methyl ethylphosphonate-b		1575
3,5-Dimethylcyclohexyl methyl propylphosphonate-a		1575
Cycloheptyl methyl ethylphosphonate		1575
Ethyl 2-methylcyclohexyl isopropylphosphonate-a		1575
1-Cyclohexylethyl ethyl methylphosphonate-b		1576
2- <i>tert</i> -Butylcyclohexyl ethylphosphonofluoridate-b		1576
<i>tert</i> -Butyldimethylsilyl 1,2-dimethylpropyl ethylphosphonate		1576
2-Cyclohexylethyl methyl methylphosphonate		1577
2,6-Dimethylcyclohexyl methyl isopropylphosphonate-b		1578
3,5-Dimethylcyclohexyl methyl isopropylphosphonate-b		1578
Ethyl 2,4,4-trimethylpentyl propylphosphonate		1578
2-Ethylcyclohexyl methyl ethylphosphonate-c		1580
Methyl 2-methylcyclohexyl propylphosphonate-a		1580
<i>tert</i> -Butyldimethylsilyl 2-methoxyethyl ethylphosphonate		1580
Ethyl 1-methyloctyl methylphosphonate-a		1581
Ethyl 2-methylcyclohexyl isopropylphosphonate-b		1581
Methyl 2-propylpentyl propylphosphonate		1581
1-Methylnonyl ethylphosphonofluoridate-b		1582
3,5-Dimethylcyclohexyl methyl isopropylphosphonate-c		1582
Ethyl 3-methylcyclohexyl isopropylphosphonate-b		1582
Ethyl octyl methylphosphonate		1582
<i>tert</i> -Butyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1582
Bis(1-ethylbutyl) methylphosphonate		1583
3-Cyclopentylpropyl methyl methylphosphonate		1584
Ethyl 2-propylpentyl isopropylphosphonate		1584
Isobutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	56217-65-3	1584
1-Butylpentyl methyl isopropylphosphonate		1585
Ethyl 3-methylcyclohexyl isopropylphosphonate-c		1585

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Compounds	CAS number	Retention index
Methyl 3-methylcyclohexyl propylphosphonate-c		1585
1-Ethylheptyl methyl ethylphosphonate-a		1586
Hexyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanide		1586
Methyl 1-methylheptyl propylphosphonate-a		1586
Methyl 2-methylcyclohexyl propylphosphonate-b		1586
Ethyl 1-propylpentyl propylphosphonate		1587
2,3-Dimethylcyclohexyl methyl ethylphosphonate-d		1588
Ethyl heptyl isopropylphosphonate		1589
1-Ethylheptyl methyl ethylphosphonate-b		1590
Ethyl 1-methylheptyl isopropylphosphonate-a		1590
Ethyl 1-methyloctyl methylphosphonate-b		1590
2,3-Dimethylcyclohexyl methyl ethylphosphonate-e		1591
Heptyl methyl propylphosphonate		1591
Methyl 1-methylheptyl propylphosphonate-b		1591
1-Cyclohexylethyl methyl ethylphosphonate-a		1592
2,6-Dimethylcyclohexyl ethyl ethylphosphonate-a		1592
2-Ethylcyclohexyl methyl isopropylphosphonate-a		1592
3,5-Dimethylcyclohexyl ethyl ethylphosphonate-b		1592
Cyclopentylmethyl ethyl propylphosphonate		1592
Methyl 3,5,5-trimethylhexyl isopropylphosphonate		1592
1,5-Dimethylhexyl ethyl propylphosphonate		1593
2-Cyclohexylethyl propylphosphonofluoridate		1593
Ethyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	21770-86-5	1594
Ethyl <i>S</i> -2-dimethylaminoethyl propylphosphonothiolate	218964-59-1	1594
Ethyl 1-methylheptyl isopropylphosphonate-b		1594
2-Ethylcyclohexyl methyl isopropylphosphonate-b		1595
Methyl 4-methylcyclohexyl propylphosphonate-b		1595
2,6-Dimethylcyclohexyl ethyl ethylphosphonate-b		1596
2- <i>tert</i> -Butylcyclohexyl ethylphosphonofluoridate-c		1596
Bis(1-methylpentyl) methylphosphonate		1596
Ethyl 4-methylcyclohexyl isopropylphosphonate-b		1596
<i>tert</i> -Butyldimethylsilyl 2-methylpentyl methylphosphonate		1596
3,5-Dimethylcyclohexyl ethyl ethylphosphonate-c		1597
4-Ethylcyclohexyl methyl ethylphosphonate-a		1597
2,3-Dimethylcyclohexyl methyl isopropylphosphonate-a		1598
3-Cyclopentylpropyl propylphosphonofluoridate		1598
Ethyl 2-ethylhexyl isopropylphosphonate		1598
Ethyl 3-methylcyclohexyl propylphosphonate-a		1598
1-Cyclohexylethyl methyl ethylphosphonate-b		1599
2- <i>tert</i> -Butylcyclohexyl ethylphosphonofluoridate-d		1599
2,2-Dimethylpropyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	56217-66-4	1600
Cyclooctyl isopropylphosphonofluoridate		1600
Ethyl 4-ethylcyclohexyl methylphosphonate-b		1600
1-Butylpentyl ethyl ethylphosphonate		1601
Cyclohexylmethyl methyl isopropylphosphonate		1601
2-Ethylcyclohexyl methyl isopropylphosphonate-c		1605
2-Ethylhexyl methyl propylphosphonate		1605
Nonyl isopropylphosphonofluoridate		1605
1,2-Dimethylpropyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		1606

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Compounds	CAS number	Retention index
3,7-Dimethyloctyl isopropylphosphonofluoridate		1606
Ethyl 3,5,5-trimethylhexyl ethylphosphonate		1607
Cycloheptyl methyl isopropylphosphonate		1608
Ethyl 1-ethylhexyl propylphosphonate-a		1608
Ethyl 2-ethylcyclohexyl ethylphosphonate-a		1608
2,3-Dimethylcyclohexyl methyl isopropylphosphonate-b		1609
Cyclooctyl methyl methylphosphonate		1611
Heptyl <i>N,N</i> -dimethylphosphoramidocyanidate	162085-91-8	1611
2-Ethylcyclohexyl methyl isopropylphosphonate-d		1612
Ethyl 1-ethylhexyl propylphosphonate-b		1612
Ethyl 4-methylcyclohexyl propylphosphonate-a		1612
Methyl 1-methyloctyl ethylphosphonate		1612
2,3-Dimethylcyclohexyl ethyl ethylphosphonate-a		1613
Methyl octyl ethylphosphonate		1613
<i>tert</i> -Butyldimethylsilyl 3-methylpentyl methylphosphonate		1613
Propyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1614
1-Ethylheptyl methyl isopropylphosphonate-a		1615
1-Methylnonyl isopropylphosphonofluoridate-a		1615
2,3-Dimethylcyclohexyl ethyl ethylphosphonate-b		1616
Cyclohexylmethyl ethyl ethylphosphonate		1616
1,2-Dimethylpropyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1618
3,5-Dimethylcyclohexyl ethyl propylphosphonate-a		1619
3-Cyclohexylpropyl ethylphosphonofluoridate		1620
Ethyl 2-methylcyclohexyl propylphosphonate-a		1620
<i>sec</i> -Butyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a	50929-96-9	1620
1-Ethylheptyl methyl isopropylphosphonate-b		1621
2,6-Dimethylcyclohexyl methyl propylphosphonate		1621
Ethyl 2-ethylcyclohexyl ethylphosphonate-b		1621
2,3-Dimethylcyclohexyl ethyl ethylphosphonate-c		1622
3,5-Dimethylcyclohexyl ethyl isopropylphosphonate-b		1622
Methyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate	170800-77-8	1622
2,3-Dimethylcyclohexyl methyl isopropylphosphonate-c		1623
1-Cyclohexylethyl methyl isopropylphosphonate-a		1624
Ethyl 2-methylcyclohexyl propylphosphonate-b		1624
2,6-Dimethylcyclohexyl ethyl isopropylphosphonate		1625
3,7-Dimethyloctyl methyl methylphosphonate		1625
Cycloheptyl ethyl ethylphosphonate		1625
3,5-Dimethylcyclohexyl methyl propylphosphonate-b		1626
Ethyl 2-propylpentyl propylphosphonate		1626
<i>sec</i> -Butyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b	50929-96-9	1626
4-Ethylcyclohexyl methyl ethylphosphonate-b		1627
Ethyl 2-ethylcyclohexyl ethylphosphonate-c		1627
1-Methylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		1628
2,3-Dimethylcyclohexyl methyl isopropylphosphonate-d		1628
Methyl nonyl methylphosphonate		1628

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Compounds	CAS number	Retention index
1-Butylpentyl ethyl isopropylphosphonate		1629
1-Butylpentyl methyl propylphosphonate		1629
3,5-Dimethylcyclohexyl ethyl isopropylphosphonate-c		1629
4-Ethylcyclohexyl methyl isopropylphosphonate-a		1629
Ethyl 3-methylcyclohexyl propylphosphonate-b		1629
1-Methylnonyl isopropylphosphonofluoride-b		1630
1-Cyclohexylethyl methyl isopropylphosphonate-b		1632
Ethyl heptyl propylphosphonate		1632
<i>O</i> -Ethyl <i>S</i> -2-ethylthioethyl methylphosphonothiolate	556-75-2	1633
2-Cyclohexylethyl ethyl methylphosphonate		1633
Ethyl 1-methylheptyl propylphosphonate		1633
2,3-Dimethylcyclohexyl ethyl ethylphosphonate-d		1634
Butyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	56217-63-1	1634
Ethyl 1-ethylheptyl ethylphosphonate		1634
1-Methylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1635
Methyl 1-methylnonyl methylphosphonate-a		1635
2-Ethylcyclohexyl methyl propylphosphonate-a		1636
Dipentyl ethylphosphonate	6163-82-2	1636
Methyl 3,5,5-trimethylhexyl propylphosphonate		1636
1-Cyclohexylethyl ethyl ethylphosphonate-a		1637
1-Ethylpropyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		1637
3,7-Dimethyloctyl propylphosphonofluoride-a		1638
Bis(1-methylpentyl) ethylphosphonate-a		1638
Methyl 1-methylnonyl methylphosphonate-b		1638
2,3-Dimethylcyclohexyl ethyl ethylphosphonate-e		1639
2-Ethylcyclohexyl methyl propylphosphonate-b		1639
Cyclooctyl propylphosphonofluoride		1639
3,7-Dimethyloctyl propylphosphonofluoride-b		1640
3-Cyclopentylpropyl ethyl methylphosphonate		1640
Ethyl 3,5,5-trimethylhexyl isopropylphosphonate		1640
Nonyl propylphosphonofluoride		1640
Ethyl 2-ethylcyclohexyl isopropylphosphonate-a		1641
2,3-Dimethylcyclohexyl ethyl isopropylphosphonate-a		1642
Butyl <i>N,N</i> -dipropylphosphoramidocyanide		1642
Ethyl 2-ethylhexyl propylphosphonate		1642
Ethyl 2-ethylcyclohexyl isopropylphosphonate-b		1643
Ethyl 4-methylcyclohexyl propylphosphonate-b		1643
Pentyl <i>N,N</i> -diisopropylphosphoramidocyanide		1643
Methyl 1-methyloctyl isopropylphosphonate		1644
Tris(2-trimethylsilyloxyethyl)amine	20836-42-4	1644
2,3-Dimethylcyclohexyl ethyl isopropylphosphonate-b		1646
Ethyl 4-ethylcyclohexyl ethylphosphonate-a		1646
1-Methylnonyl propylphosphonofluoride-a		1647
2,3-Dimethylcyclohexyl methyl propylphosphonate-a		1647
Cyclohexylmethyl methyl propylphosphonate		1647
Methyl octyl isopropylphosphonate		1647
2- <i>tert</i> -Butylcyclohexyl propylphosphonofluoride-a		1649
Cyclohexylmethyl ethyl isopropylphosphonate		1649
1-Cyclohexylethyl ethyl ethylphosphonate-b		1650

(continued)

Compounds	CAS number	Retention index
Bis(1-methylpentyl) ethylphosphonate-b		1650
<i>tert</i> -Butyldimethylsilyl hexyl methylphosphonate		1651
Ethyl 2-ethylcyclohexyl isopropylphosphonate-c		1652
2,3-Dimethylcyclohexyl methyl propylphosphonate-b		1654
2-Ethylcyclohexyl methyl propylphosphonate-c		1655
Cycloheptyl methyl propylphosphonate		1655
Isobutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1655
2,3-Dimethylcyclohexyl ethyl isopropylphosphonate-c		1656
Cycloheptyl ethyl isopropylphosphonate		1656
Ethyl 1-methyloctyl ethylphosphonate-a		1656
Ethyl octyl ethylphosphonate		1656
1-Ethylheptyl methyl propylphosphonate-a		1657
2- <i>tert</i> -Butylcyclohexyl propylphosphonofluoride-b		1657
2-Ethylcyclohexyl methyl propylphosphonate-d		1658
Decyl ethylphosphonofluoride		1658
Dicyclopentyl methylphosphonate	22583-27-3	1658
Ethyl 2-ethylcyclohexyl isopropylphosphonate-d		1658
2-Cyclohexylethyl methyl ethylphosphonate		1659
Bis(3,3-dimethylbutyl) ethylphosphonate		1659
Bis(1-methylpentyl) ethylphosphonate-c		1661
Ethyl 1-ethylheptyl isopropylphosphonate-a		1661
Ethyl 1-methyloctyl ethylphosphonate-b		1661
Pinacolyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a	34388-36-8	1663
1-Ethylheptyl methyl propylphosphonate-b		1664
Cyclooctyl ethyl methylphosphonate	22583-40-0	1664
Ethyl 1-ethylheptyl isopropylphosphonate-b		1664
1-Methylnonyl propylphosphonofluoride-b		1665
2,6-Dimethylcyclohexyl ethyl propylphosphonate-a		1665
4-Ethylcyclohexyl methyl isopropylphosphonate-b		1666
Hexyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanide		1666
2,3-Dimethylcyclohexyl ethyl isopropylphosphonate-d		1667
1-Cyclohexylethyl ethyl isopropylphosphonate-a		1668
3-Cyclopentylpropyl methyl ethylphosphonate		1668
Methyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	198830-32-9	1668
1-Butylpentyl ethyl propylphosphonate		1669
3,5-Dimethylcyclohexyl ethyl propylphosphonate-b		1669
2,3-Dimethylcyclohexyl methyl propylphosphonate-c		1670
3-Cyclohexylpropyl isopropylphosphonofluoride		1670
Ethyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate	21738-25-0	1671
2,6-Dimethylcyclohexyl ethyl propylphosphonate-b		1672
1-Cyclohexylethyl methyl propylphosphonate		1673
2,2-Dimethylpropyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1674
2,3-Dimethylcyclohexyl ethyl isopropylphosphonate-e		1674
3,5-Dimethylcyclohexyl ethyl propylphosphonate-c		1675
4-Ethylcyclohexyl methyl propylphosphonate-a		1675
3,7-Dimethyloctyl ethyl methylphosphonate		1676
Ethyl 4-ethylcyclohexyl isopropylphosphonate-a		1676

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Compounds	CAS number	Retention index
3-Methylbutyl S-2-dimethylaminoethyl methylphosphonothiolate		1676
Ethyl 4-ethylcyclohexyl ethylphosphonate-b		1678
1,2-Dimethylpropyl S-2-dimethylaminoethyl ethylphosphonothiolate-a		1679
2-tert-Butylcyclohexyl propylphosphonofluoridate-c		1680
Cyclopropylmethyl S-2-dimethylaminoethyl methylphosphonothiolate		1680
Ethyl 1-methylnonyl methylphosphonate-a		1680
Ethyl 2-ethylcyclohexyl propylphosphonate-a		1680
Isopropyl S-2-diethylaminoethyl ethylphosphonothiolate	162085-93-0	1680
Ethyl 3,5,5-trimethylhexyl propylphosphonate		1681
Ethyl nonyl methylphosphonate		1681
1-Cyclohexylethyl ethyl isopropylphosphonate-b		1682
2-Methylbutyl S-2-dimethylaminoethyl methylphosphonothiolate		1682
Heptyl N-ethyl-N-methylphosphoramidocyanidate		1682
Ethyl 2-ethylcyclohexyl propylphosphonate-b		1683
Pinacolyl S-2-dimethylaminoethyl methylphosphonothiolate-b	34388-36-8	1683
Propyl S-2-dimethylaminoethyl propylphosphonothiolate		1683
Methyl 1-methyloctyl propylphosphonate-a		1684
2-tert-Butylcyclohexyl propylphosphonofluoridate-d		1687
Ethyl 1-methyloctyl isopropylphosphonate-a		1687
Ethyl octyl isopropylphosphonate		1687
1,2-Dimethylpropyl S-2-dimethylaminoethyl ethylphosphonothiolate-b		1689
Ethyl 1-methylnonyl methylphosphonate-b		1689
Methyl 1-methyloctyl propylphosphonate-b		1689
2,3-Dimethylcyclohexyl ethyl propylphosphonate-a		1690
Ethyl 1-methyloctyl isopropylphosphonate-b		1690
Methyl octyl propylphosphonate		1691
2-Cyclohexylethyl methyl isopropylphosphonate		1693
3-Cyclohexylpropyl methyl methylphosphonate		1693
2,3-Dimethylcyclohexyl ethyl propylphosphonate-b		1694
Cyclohexylmethyl ethyl propylphosphonate		1694
Cyclooctyl methyl ethylphosphonate		1694
Ethyl 2-ethylcyclohexyl propylphosphonate-c		1698
2,3-Dimethylcyclohexyl ethyl propylphosphonate-c		1699
1-Methylbutyl S-2-dimethylaminoethyl ethylphosphonothiolate-a		1700
3-Cyclopentylpropyl methyl isopropylphosphonate		1700
3,7-Dimethyloctyl methyl ethylphosphonate		1701
Butyl S-2-dimethylaminoethyl ethylphosphonothiolate		1701
Cycloheptyl ethyl propylphosphonate		1701
Ethyl 1-ethylheptyl propylphosphonate-a		1701
Ethyl 2-ethylcyclohexyl propylphosphonate-d		1701
O,O-Diethyl S-2-diethylaminoethyl phosphorothiolate	78-53-5	1703
1,2-Bis(2-chloroethylthio)ethane	3563-36-8	1703
1,2-Dimethylbutyl S-2-dimethylaminoethyl methylphosphonothiolate-a		1704

(continued)

Compounds	CAS number	Retention index
Ethyl 1-ethylheptyl propylphosphonate-b		1704
1-Methylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		1705
Isopropyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	51446-23-2	1705
3-Cyclohexylpropyl propylphosphonofluoridate		1706
Methyl nonyl ethylphosphonate		1706
Decyl isopropylphosphonofluoridate		1707
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1709
1-Ethylpropyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1709
2-Cyclohexylethyl ethyl ethylphosphonate		1710
1-Ethylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		1711
2,3-Dimethylcyclohexyl ethyl propylphosphonate-d		1711
4-Ethylcyclohexyl methyl propylphosphonate-b		1712
Ethyl 4-ethylcyclohexyl isopropylphosphonate-b		1712
Methyl 1-methylnonyl ethylphosphonate		1712
1-Cyclohexylethyl ethyl propylphosphonate-a		1713
Bis(2-ethylbutyl) ethylphosphonate		1713
Ethyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	50782-69-9	1713
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-c		1714
Bis(2-methylpentyl) ethylphosphonate		1714
2,3-Dimethylcyclohexyl ethyl propylphosphonate-e		1715
3-Cyclopentylpropyl ethyl ethylphosphonate		1717
1-Methylpentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a	79351-19-2	1718
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-d		1719
Dicyclopentyl ethylphosphonate		1720
Ethyl 4-ethylcyclohexyl propylphosphonate-a		1720
1-Cyclohexylethyl ethyl propylphosphonate-b		1721
1-Methylpentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b	79351-19-2	1726
Bis(3,3-dimethylbutyl) propylphosphonate		1728
Cyclooctyl methyl isopropylphosphonate		1728
Pentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	56217-64-2	1728
Decyl methyl methylphosphonate		1729
Ethyl 1-methyloctyl propylphosphonate		1730
Ethyl octyl propylphosphonate		1731
Isobutyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	159939-87-4	1732
3,7-Dimethyloctyl methyl isopropylphosphonate		1735
<i>tert</i> -Butyldimethylsilyl cyclohexyl methylphosphonate		1736
Pinacolyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		1738
2-Cyclohexylethyl methyl propylphosphonate		1739
Isopropyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1739
Pentyl <i>N,N</i> -dipropylphosphoramidocyanidate		1739

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Compounds	CAS number	Retention index
Methyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate	162085-94-1	1741
Methyl nonyl isopropylphosphonate		1741
2-Cyclohexylethyl ethyl isopropylphosphonate		1742
Cyclooctyl ethyl ethylphosphonate		1742
Decyl propylphosphonofluoridate		1742
Methyl 1-methylnonyl isopropylphosphonate-a		1743
Propyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1744
2,2-Dimethylpropyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		1745
Methyl 1-methylnonyl isopropylphosphonate-b		1745
3,7-Dimethyloctyl ethyl ethylphosphonate		1747
3-Cyclopentylpropyl methyl propylphosphonate		1747
3-Methylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1747
3-Cyclohexylpropyl ethyl methylphosphonate		1748
3-Cyclopentylpropyl ethyl isopropylphosphonate		1749
1,2-Dimethylpropyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-a		1751
Bis(4-methylpentyl) ethylphosphonate		1753
Ethyl 1-methylnonyl ethylphosphonate-a		1753
Bis(3-methylpentyl) ethylphosphonate		1754
Cyclopropylmethyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1755
Ethyl nonyl ethylphosphonate	13274-95-8	1755
2-Methylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1756
Pinacolyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		1756
Ethyl 1-methylnonyl ethylphosphonate-b		1757
<i>sec</i> -Butyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-a		1757
Ethyl 4-ethylcyclohexyl propylphosphonate-b		1758
1,2-Dimethylpropyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-b		1761
<i>sec</i> -Butyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-b		1762
Heptyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanidate		1763
1-Methylbutyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-a		1764
2-Methylpentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		1766
1-Methylbutyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-b		1770
1-Ethylpropyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		1772
3-Cyclohexylpropyl methyl ethylphosphonate		1773
Cyclooctyl ethyl isopropylphosphonate		1774
Cyclopentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	22925-98-0	1775
3,7-Dimethyloctyl methyl propylphosphonate		1776
Cyclooctyl methyl propylphosphonate		1776
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		1777
3,7-Dimethyloctyl ethyl isopropylphosphonate		1779

(continued)

Compounds	CAS number	Retention index
Bis(2-ethylbutyl) propylphosphonate		1779
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		1781
Decyl ethyl methylphosphonate		1781
1-Ethylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1782
Bis(2-methylpentyl) propylphosphonate		1782
Methyl 1-methylnonyl propylphosphonate-a		1782
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-c		1783
Methyl nonyl propylphosphonate		1784
Ethyl 1-methylnonyl isopropylphosphonate-a		1785
Propyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	52364-45-1	1785
Ethyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate	73835-17-3	1786
Octyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanide		1786
2-Cyclohexylethyl ethyl propylphosphonate		1787
Ethyl nonyl isopropylphosphonate		1787
<i>tert</i> -Butyldimethylsilyl 2-methylcyclohexyl methylphosphonate		1787
1,2-Dimethylbutyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-d		1788
Ethyl 1-methylnonyl isopropylphosphonate-b		1788
Methyl 1-methylnonyl propylphosphonate-b		1788
1-Methylpentyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		1789
Isobutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1789
Isopropyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate	162085-95-2	1789
1-Methylpentyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		1794
3-Cyclopentylpropyl ethyl propylphosphonate		1794
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		1795
<i>tert</i> -Butyldimethylsilyl 2-ethylhexyl methylphosphonate		1795
Pentyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1799
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		1801
2-Methylcyclopentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		1802
2-Methylcyclopentyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1805
Decyl methyl ethylphosphonate		1806
Isopropyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1806
Ethyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate		1809
Isopropyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate		1809
3-Cyclohexylpropyl methyl isopropylphosphonate		1810
1,3-Bis(2-chloroethylthio)propane	63905-10-2	1811

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Compounds	CAS number	Retention index
Pinacolyl S-2-diethylaminoethyl methylphosphonothiolate-a		1812
<i>tert</i> -Butyldimethylsilyl cyclohexyl ethylphosphonate		1813
2,2-Dimethylpropyl S-2-diethylaminoethyl ethylphosphonothiolate		1816
Propyl S-2-dipropylaminoethyl methylphosphonothiolate		1817
2-Methylbutyl S-2-diethylaminoethyl methylphosphonothiolate		1818
1,2-Dimethylpropyl S-2-diethylaminoethyl ethylphosphonothiolate-a		1820
Cyclooctyl ethyl propylphosphonate		1820
3,7-Dimethyloctyl ethyl propylphosphonate		1821
3-Methylbutyl S-2-diethylaminoethyl methylphosphonothiolate		1822
3-Cyclohexylpropyl ethyl ethylphosphonate		1823
Bis(3-methylpentyl) propylphosphonate		1823
Bis(4-methylpentyl) propylphosphonate		1823
Ethyl 1-methylnonyl propylphosphonate		1827
Propyl S-2-diethylaminoethyl propylphosphonothiolate		1827
Dihexyl ethylphosphonate	6151-92-4	1828
Hexyl S-2-dimethylaminoethyl methylphosphonothiolate	69777-00-0	1828
Isobutyl S-2-diisopropylaminoethyl methylphosphonothiolate		1828
1,2-Dimethylpropyl S-2-diethylaminoethyl ethylphosphonothiolate-b		1830
Pinacolyl S-2-diethylaminoethyl methylphosphonothiolate-b		1830
<i>sec</i> -Butyl S-2-dipropylaminoethyl methylphosphonothiolate-a		1830
Ethyl nonyl propylphosphonate		1831
1-Methylbutyl S-2-diethylaminoethyl ethylphosphonothiolate-a		1833
<i>sec</i> -Butyl S-2-dipropylaminoethyl methylphosphonothiolate-b		1835
Butyl S-2-diethylaminoethyl ethylphosphonothiolate		1836
1-Methylbutyl S-2-diethylaminoethyl ethylphosphonothiolate-b		1837
1,2-Dimethylbutyl S-2-diethylaminoethyl methylphosphonothiolate-a		1839
2-Methylpentyl S-2-dimethylaminoethyl ethylphosphonothiolate		1839
1-Ethylpropyl S-2-diethylaminoethyl ethylphosphonothiolate		1840
Decyl methyl isopropylphosphonate		1841
1,2-Dimethylbutyl S-2-diethylaminoethyl methylphosphonothiolate-b		1843
1-Ethylbutyl S-2-diethylaminoethyl methylphosphonothiolate		1843
Cyclopentyl S-2-dimethylaminoethyl ethylphosphonothiolate		1844
1,2-Dimethylbutyl S-2-diethylaminoethyl methylphosphonothiolate-c		1847

(continued)

Compounds	CAS number	Retention index
1,2-Dimethylbutyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-d		1852
1-Methylpentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-a		1853
Propyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1853
2,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		1854
3-Cyclohexylpropyl methyl propylphosphonate		1855
Decyl ethyl ethylphosphonate		1855
3-Cyclohexylpropyl ethyl isopropylphosphonate		1857
1,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		1859
1-Methylpentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-b		1859
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		1861
Isobutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1862
2-Methylcyclopentyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		1865
Pentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		1865
Octyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanidate		1866
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		1866
2-Methylcyclopentyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		1868
1,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		1870
<i>O</i> -Ethyl <i>S</i> -nonyl methylphosphonothiolate	13088-89-6	1872
Pinacolyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-a		1873
1-Methylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		1874
Cyclohexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate	69795-06-8	1874
1-Ethylpropyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		1880
1-Methylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		1880
Decyl methyl propylphosphonate		1885
Nonyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanidate		1885
Decyl ethyl isopropylphosphonate		1886
2-Methylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1887
3-Methylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1887
Propyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1887
2,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1888
Pinacolyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-b		1889

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(continued)

Compounds	CAS number	Retention index
Butyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	52364-46-2	1891
<i>sec</i> -Butyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		1894
1,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		1895
Isobutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1895
Diethyl propylphosphonate		1896
<i>sec</i> -Butyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		1899
2-Methylpentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		1900
Hexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1900
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate-a		1902
3-Cyclohexylpropyl ethyl propylphosphonate		1903
1,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		1905
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate-b		1906
1-Methylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		1907
Pinacolyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a	198830-38-5	1907
1,2-Dimethylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-a		1908
1-Ethylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1908
Bis(trimethylsilyl)benzilate	126298-91-7	1908
Butyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1911
1,2-Dimethylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-b		1912
Cyclopentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	93240-66-5	1912
1-Methylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		1914
1,2-Dimethylbutyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-c		1916
1-Ethylpropyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1916
1-Methylpentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-a		1920
2-Methylcyclohexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		1920
2,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1921
1-Methylpentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-b		1923
1,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		1924
Pinacolyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b	198830-38-5	1925

(continued)

Compounds	CAS number	Retention index
2-Methylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		1930
2-Methylcyclohexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		1930
2-Methylcyclopentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-a		1930
Decyl ethyl propylphosphonate		1930
Isobutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1930
4-Methylpentyl <i>S</i> -2-dimethylaminoethyl propylphosphonothiolate		1931
3-Methylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		1932
Dicyclohexyl ethylphosphonate	169739-47-3	1932
Heptyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		1932
Pentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1932
1,2-Dimethylpropyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		1933
1,4-Bis(2-chloroethylthio)butane	142868-93-7	1933
2-Methylcyclopentyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate-b		1933
1-Methylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		1937
Propyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate		1939
1-Methylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		1940
1-Ethylpropyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1942
Pinacolyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		1942
Butyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1945
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate-a		1945
2-Ethylhexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		1947
Cyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		1949
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		1950
<i>sec</i> -Butyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate-b		1950
1-Ethylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		1951
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		1953
2,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1955
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-c		1958
1,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		1960

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(continued)

Compounds	CAS number	Retention index
1-Methylpentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		1960
Pinacolyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		1960
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-d		1963
Hexyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		1963
2-Methylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1964
3-Methylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1965
1-Methylpentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		1966
Nonyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanidate		1966
2-Methylpentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1967
1,2-Dimethylpropyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		1968
1-Methylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		1970
Propyl <i>S</i> -2-dipropylaminoethyl propylphosphonothiolate		1970
1-Methylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		1975
Pinacolyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		1975
Pentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	198830-36-3	1976
Butyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1977
Cyclopentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		1978
1-Ethylpropyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		1979
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		1981
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		1982
1-Ethylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		1982
Butyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate		1982
Bis(2-ethylhexyl) methylphosphonate	60556-68-5	1986
Decyl <i>N</i> -ethyl- <i>N</i> -methylphosphoramidocyanidate		1986
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-c		1989
Bis(2-chloroethylthioethyl)ether	63918-89-8	1990
1-Methylpentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		1991
Pinacolyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		1991
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-d		1993
2-Methylcyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		1994

(continued)

Compounds	CAS number	Retention index
2-Methylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1995
2-Methylcyclopentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-a		1996
3-Methylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		1996
1-Methylpentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		1997
2-Methylcyclopentyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate-b		2000
2-Methylcyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		2002
Heptyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		2003
2-Methylpentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		2008
Pentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2008
Pinacolyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		2010
1-Ethylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2012
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		2013
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		2016
2-Ethylhexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		2016
1,2-Dimethylbutyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-c		2020
1-Methylpentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		2022
1-Methylpentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		2025
1-Ethylpropyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate		2026
Cyclohexyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate	71293-89-5	2026
Pinacolyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		2026
2-Methylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2027
Butyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate		2028
2-(2-Chlorovinyl)-5-methyl-1,3,2-benzodithiarsole		2029
3-Methylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2029
Hexyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		2031
Octyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		2033
2-Methylpentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2040
Pentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2040

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(continued)

Compounds	CAS number	Retention index
2-Methylcyclopentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-a		2044
1-Ethylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2045
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		2049
2-Methylcyclopentyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate-b		2049
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		2053
1-Methylpentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		2055
1,2-Dimethylbutyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-c		2057
1-Methylpentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		2060
Cyclopentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2060
Heptyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		2065
Decyl <i>N</i> -methyl- <i>N</i> -propylphosphoramidocyanidate		2070
Pentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2071
Hexyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	82721-31-1	2072
2-Methylcyclopentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-a		2075
2-Methylpentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2075
2-Methylcyclopentyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate-b		2079
Cyclohexyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		2080
Cyclopentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2089
2-Methylcyclopentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-a		2102
Hexyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2103
Octyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		2103
2-Methylcyclopentyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate-b		2105
2-Methylpentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2106
Cyclopentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2122
3,5,5-Trimethylhexyl <i>S</i> -2-dimethylaminoethyl propylphosphonothiolate		2123
Cyclohexyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		2124
Heptyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		2130
Hexyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2136

(continued)

Compounds	CAS number	Retention index
Nonyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		2136
2-Methylcyclopentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-a		2137
2-Methylcyclopentyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate-b		2141
Cyclohexyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2158
5-Isopropyl-2-methylcyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		2160
Octyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		2167
5-Isopropyl-2-methylcyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-b		2168
Hexyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2170
Octyl <i>S</i> -2-dimethylaminoethyl propylphosphonothiolate		2171
Heptyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		2173
4-Methylpentyl <i>S</i> -2-diisopropylaminoethyl propylphosphonothiolate		2179
Cyclohexyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2189
Heptyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2203
Nonyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		2206
Cyclohexyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2224
Octyl <i>S</i> -2-diethylaminoethyl ethylphosphonothiolate		2232
Heptyl <i>S</i> -2-diisopropylaminoethyl ethylphosphonothiolate		2237
4- <i>tert</i> -Butylcyclohexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-a		2241
4- <i>tert</i> -Butylcyclohexyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate-b		2241
Decyl <i>S</i> -2-dimethylaminoethyl methylphosphonothiolate		2241
Heptyl <i>S</i> -2-dipropylaminoethyl ethylphosphonothiolate		2269
Nonyl <i>S</i> -2-diethylaminoethyl methylphosphonothiolate		2269
Heptyl <i>S</i> -2-diisopropylaminoethyl isopropylphosphonothiolate		2275
Octyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate		2276
Octyl <i>S</i> -2-dipropylaminoethyl methylphosphonothiolate		2303
Decyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate		2309
4- <i>tert</i> -Butylcyclohexyl <i>S</i> -2-dimethylaminoethyl ethylphosphonothiolate-a		2313

(continued overleaf)

(continued)

Compounds	CAS number	Retention index
4- <i>tert</i> -Butylcyclohexyl S-2-dimethylaminoethyl ethylphosphonothiolate-b		2313
Nonyl S-2-diethylaminoethyl ethylphosphonothiolate		2331
Octyl S-2-diisopropylaminoethyl ethylphosphonothiolate		2338
Cyclohexylmethyl S-2-diisopropylaminoethyl isopropylphosphonothiolate		2364
Octyl S-2-dipropylaminoethyl ethylphosphonothiolate		2368
3,5,5-Trimethylhexyl S-2-diisopropylaminoethyl propylphosphonothiolate		2370
Decyl S-2-diethylaminoethyl methylphosphonothiolate		2371
Nonyl S-2-diisopropylaminoethyl methylphosphonothiolate		2377
Nonyl S-2-dipropylaminoethyl methylphosphonothiolate		2404
Cyclohexylmethyl S-2-diisopropylaminoethyl propylphosphonothiolate		2410
Decyl S-2-diethylaminoethyl ethylphosphonothiolate		2434
Nonyl S-2-diisopropylaminoethyl ethylphosphonothiolate		2441
Nonyl S-2-dipropylaminoethyl ethylphosphonothiolate		2469
Decyl S-2-diisopropylaminoethyl methylphosphonothiolate		2480
Decyl S-2-dipropylaminoethyl methylphosphonothiolate		2508
Decyl S-2-diisopropylaminoethyl ethylphosphonothiolate		2542
Decyl S-2-dipropylaminoethyl ethylphosphonothiolate		2570
3-Quinuclidinyl benzilate trimethylsilyl ether		2633
3-Quinuclidinyl benzilate	6581-06-2	2658

-a,-b,-c,-d: A diastereomeric compound gives more than one peak

-ar,-br,-cr: A racemic mixture

CHAPTER 11

Gas Chromatography/Mass Spectrometry in Analysis of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Analytical measurements play a role in underpinning the elements of the verification regime of

the Chemical Weapons Convention (CWC). It is difficult to conceive of circumstances where any claim of noncompliance under the Convention could be sustained without sound analytical evidence.

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

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GC/MS (Gas chromatography/mass spectrometry) is at present the most suitable technique for the analysis of chemicals related to the CWC, as it is capable of providing the required analytical evidence. The provisions for the collection, handling, and analysis of samples during an inspection are laid down in paragraphs 52–58 of Part II of the Verification Annex of the Convention text ⁽¹⁾. The Convention addresses the matter of security, integrity, and preservation of samples as well as the confidentiality aspects of the analysis. Special attention is also paid to quality assurance (QA). The collection of samples and the analysis of those samples should be focused on the aims of the inspection, that is, to gain information relevant to the verification of compliance with the Convention. During inspections at facilities where activities not prohibited by the Convention take place, a balance in analysis must be kept between the need to verify the presence or absence of scheduled chemicals on the one hand, and, on the other hand, the requirement that information not relevant to the CWC should not be revealed. Depending on the inspection scenario, samples shall be taken either by representatives of the inspected facility in the presence of inspectors or by the inspection team itself. Chemical analysis can be carried out on-site, during an inspection, or off-site, in at least two designated laboratories selected by the Technical Secretariat of the OPCW (Organization for the Prohibition of Chemical Weapons). It is clearly stated in the Convention text that, where possible, the analysis of inspection samples shall be performed on site. Generally, an on-site laboratory will be small and equipped only with the most essential means, such as a sample preparation kit and a mobile GC/MS instrument. In the event that ambiguities arise during an inspection, the Convention offers the possibility to perform the analysis off site in designated laboratories equipped with more sophisticated GC/MS instrumentation. In view of the possible political impact of verification analysis, the results reported by an off-site laboratory should be absolutely certain, and false positive results are unacceptable. Therefore, a set of identification criteria has to be fulfilled. In principle, these criteria are based on the fact that at least two independent analytical techniques have to be used leading to consistent positive identification results. The two most applied techniques in the verification analysis

are low-resolution electron impact (EI) and chemical ionization (CI) GC/MS under full scan conditions.

The chemicals placed on the CWC Schedule list are the target for the verification analysis, especially the Schedule 1 chemicals that encompass the well-known chemical warfare (CW) agents, such as the nerve agents sarin, soman, tabun, and VX (*O*-ethyl *S*-2-diisopropylaminoethyl methylphosphonothiolate), and the vesicants, mustard gas and lewisite 1 (see Table 1). Incapacitants such as ω -chloroacetophenone (code CN, CAS 532-27-4) and *o*-chlorobenzylidenemalononitrile (code CS, CAS 2698-41-1), which have been developed in the past as military tear gases, are not considered in this contribution. The toxic Schedule 1 chemicals, grouped under Schedule 1.A, consist of a specific number of vesicants and toxins, and several families of compounds. The first family (Schedule 1.A.1) encompasses the homologous series of alkyl and cycloalkyl (alkyl/cycloalkyl \leq C10) alkyl(methyl, ethyl, isopropyl or propyl)phosphonofluoridates, of which the family of alkyl and cycloalkyl methylphosphonofluoridates is the most important. Sarin (alkyl: isopropyl) and soman (alkyl: 1,2,2-trimethylpropyl) belong to this family. The trivial name sarin is sometimes used as the reference compound name, for example, cyclohexyl sarin (GF) instead of cyclohexyl methylphosphonofluoridate (see Table 1). The second family concerns *O*-alkyl/cycloalkyl *N,N*-dialkyl (methyl, ethyl, isopropyl or propyl) phosphoramidocyanidates (Schedule 1.A.2). These compounds are related to tabun (see Table 1). The third family concerns *O*-alkyl/cycloalkyl (H, \leq C10) *S*-2-dialkyl (methyl, ethyl, isopropyl, propyl) aminoethyl alkyl (methyl, ethyl, isopropyl, propyl) phosphonothiolates (Schedule 1.A.3). These compounds are related to VX (see Table 1). In principle, the number of substituent permutations in this family is even greater than in the case of the sarin and tabun families.

Analysis under the Convention is focused primarily on qualitative analysis (unambiguous identification) rather than on quantitative analysis. For the presence or absence of Schedule 1 chemicals, no quantitative limits are set in the Convention. Sample preparation methods for GC/MS proceed with a certain efficiency, and for accurate quantitative analysis, a determination of the recovery efficiency will be necessary. This may be performed by spiking a blank background sample and carrying out

Table 1. Chemical information and GC/MS literature references for some important CW agents and related compounds placed on the CWC Schedule list

Systematical name	Code/trivial name	CAS no.	Schedule	MS refs
Isopropyl methylphosphonofluoridate	GB/sarin	107-44-8	1.A.1	2, 5
1,2,2-Trimethylpropyl methylphosphonofluoridate	GD/soman	96-64-0	1.A.1	2, 5, 10
Cyclohexyl methylphosphonofluoridate	GF/cyclohexyl sarin	329-99-7	1.A.1	5
Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate	GA/tabun	77-81-6	1.A.2	2, 5, 13
<i>O</i> -Ethyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate	VX	50782-69-9	1.A.3	2, 3, 5
Bis(2-chloroethyl)sulfide	HD/mustard gas	505-60-2	1.A.4	6, 9, 11
1,2-Bis(2-chloroethylthio)ethane	Q/sesquimustard	3563-36-8	1.A.4	6, 9
2-Chlorovinyl dichloroarsine	L 1/lewisite 1	541-25-3	1.A.5	6, 9
Bis(2-chlorovinyl)chloroarsine	L 2/lewisite 2	40334-69-8	1.A.5	6
Tris(2-chlorovinyl)arsine	L 3/lewisite 3	40334-70-1	1.A.5	6
Bis(2-chloroethyl)methylamine	HN-2/methyl nitrogen mustard	51-75-2	1.A.6	6
Bis(2-chloroethyl)ethylamine	HN-1/ethyl nitrogen mustard	538-07-8	1.A.6	6
Tris(2-chloroethyl)amine	HN-3/nitrogen mustard	555-77-1	1.A.6	6, 9
Methylphosphonyldifluoride	DF	676-99-3	1.B.9	4, 8
<i>O</i> -Ethyl <i>O</i> -2-diisopropylaminoethylphosphonite	QL	57856-11-8	1.B.10	4, 8
Isopropyl methylphosphonochloridate	Chlorosarin	1445-76-7	1.B.11	4
1,2,2-Trimethylpropyl methylphosphonochloridate	Chlorosoman	7040-57-5	1.B.12	4, 10
<i>O,O</i> -Diethyl <i>S</i> -2-diethylaminoethyl phosphorothiolate	Amiton	78-53-5	2.A.1	
1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene	PFIB	382-21-8	2.A.2	
3-Quinuclidinyl benzilate	BZ	6581-06-2	2.A.3	6, 12
Dimethyl methylphosphonate	DMMP	756-79-6	2.B.4	2, 4, 7
Diisopropyl methylphosphonate	DIMP	1445-75-6	2.B.4	2, 4
Methylphosphonyl dichloride	—	676-97-1	2.B.4	4, 8
Methylthiophosphonyl dichloride	—	676-98-2	2.B.4	4
<i>N,N</i> -Dimethylphosphoramidic dichloride	—	677-43-0	2.B.5	4
Diethyl <i>N,N</i> -dimethylphosphoramidate	—	2404-03-7	2.B.6	4, 13
Arsenic trichloride	—	7784-34-1	2.B.7	4
2,2-Diphenyl-2-hydroxyacetic acid	Benzilic acid	76-93-7	2.B.8	4, 8
Quinuclidine-3-ol	—	1619-34-7	2.B.9	4, 8
<i>N,N</i> -Diisopropylaminoethyl-2-chloride	—	96-79-7	2.B.10	4
<i>N,N</i> -Diisopropylaminoethane-2-ol	—	96-80-0	2.B.11	4
<i>N,N</i> -Diisopropylaminoethane-2-thiol	—	5842-07-9	2.B.12	3, 4
Bis(2-hydroxyethyl)sulfide	Thiodiglycol	111-48-8	2.B.13	4, 8, 11
3,3-Dimethylbutane-2-ol	Pinacolyl alcohol	464-07-3	2.B.14	4, 8

the same sample preparation and GC/MS analysis method on the spiked sample. However, background samples and spiking chemicals are not always available. Moreover, accurate quantitative results are only valid when stable analytes have to be determined, but some CW agents are certainly not stable in environmental samples. Although quantitative analysis may not be the prime aim, sample preparation and GC/MS analysis methods should proceed with sufficient efficiency to allow the determination of the compounds of CW interest at a reasonably low level (e.g. $<1 \text{ mg kg}^{-1}$) in environmental samples.

The analysis of scheduled compounds causes several problems owing to the large variety of these compounds. On the CWC Schedule list, very volatile chemicals, such as the blood gases hydrogen cyanide (HCN, CAS 74-90-8) and phosgene (CAS 75-44-5) are placed along the very polar, nonvolatile toxins saxitoxin (CAS 35523-89-8) and ricin (CAS 9009-86-3). In addition to the common organic CHNO-containing compounds, a great variety of other compounds need to be considered. This encompasses organic phosphorus-, sulfur-, arsenic-, chlorine-, and fluorine-containing chemicals, some of which are quite reactive and, hence, unstable in environmental matrices. It is virtually impossible for all these compounds of interest to the Convention to be determined with the highest efficiency by a few general sample preparation and GC/MS analysis methods. Moreover, saxitoxin and ricin cannot be determined by GC/MS, but require analysis by liquid chromatographic (LC) and other mass spectrometric techniques (e.g. electrospray (ES) ionization, LC/ES/MS). Depending on the type of inspection, analyses may have to be directed toward the declaration and/or the type of facility. This will be the case, in particular, when biomedical samples have to be considered, resulting from an investigation of an alleged use of chemical weapons.

2 ELECTRON IMPACT MASS SPECTROMETRY OF CHEMICAL WEAPONS CONVENTION SCHEDULED COMPOUNDS

2.1 General

EI is the oldest and still the most used ionization technique for the analysis of CWC-related chemicals.

All major mass spectral data collections consist of EI mass spectra, mostly recorded under accepted standardized conditions such as an ionization voltage of 70 eV, an emission current of 100–200 μA , and an ion source temperature of 150–200 °C. Several types of GC/MS systems may be applied, for instance, magnetic sector, quadrupole, or ion trap analyzers. Ion trap systems are considered less applicable, when data comparison is required with spectra from a reference library. In particular, basic compounds related to VX or the three nitrogen mustards tend to produce protonated molecular ions by self-protonation. Magnetic sector and quadrupole mass spectrometers suffer less from interference of self-protonation, and spectra produced with these types of instruments are generally reproducible.

Various classes of CW agents and related compounds have been studied by EI/MS. Research on CW agents normally takes place in specialized chemical defense institutes capable of handling the extremely toxic materials. However, work in this area is sometimes restricted and not all information has been submitted for publication, particularly in the past. EI mass spectra of the most well-known nerve agents were published for the first time in 1979 ⁽²⁾, and a vast amount of papers on GC/MS analysis of CW agents and related compounds has appeared in the scientific literature since then. Table 1 refers to the main papers dealing with EI mass spectral data and description of the fragmentation processes of CWC-related chemicals. MS of organophosphorus CW agents was reviewed briefly in 1996 as part of a general review of the chemistry of organophosphorus CW agents ⁽¹⁴⁾. EI mass spectral data on 50 possible precursors for the production of CW agents have been presented in the form of an eight-peak index ⁽⁴⁾. Several reports (so-called Blue Books) were published, between 1979 and 1986, containing mass spectrometric and gas chromatographic data of compounds of CW interest by the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN). The compilations, in the form of atlases (available upon request), encompass EI and CI mass spectra of organophosphorus ⁽⁵⁾ and nonphosphorus CW agents ⁽⁶⁾, and of some degradation products and precursors of these agents ^(7,8). The fragmentation of organophosphorus esters, including a number of possible nerve agent precursors, was extensively reviewed in 1976 ⁽¹⁵⁾. EI mass spectra of some simple scheduled chemicals

used for the production of CW agents, such as pinacolyl alcohol and thiodiglycol (see Table 1), are found in commercially available mass spectral databases.

The major source today for reference data on CWC-related chemicals is the OPCW Analytical Database ⁽¹⁶⁾. This database is being created through contributions of laboratories specialized in the analysis of CWC-related chemicals. Each contribution is validated by experts in accordance with prescribed rules. In principle, this database will contain all essential data for the identification of chemicals relevant to the Convention. Reference data obtained by various analytical techniques need to be compiled. In practice, this database will encompass GC retention indices (RIs), EI mass spectra, infrared (IR) spectra, and nuclear magnetic resonance (NMR) spectral data in addition to the chemical information data (trivial and systematic name, CAS number if assigned, Schedule number, structural composition, elemental composition, and molecular weight (MW)). It is an enormous task to collect analytical data on all chemicals relevant to the Convention as represented in the families containing all possible members of the alkyl/cycloalkyl moiety with up to 10 carbon atoms. Although the database is not complete, it is continuously being expanded and contains the EI mass spectra of all well-known CW agents and most of their degradation products. The fragmentation under EI conditions of a number of chemicals belonging to the CWC Schedule list will be discussed briefly below.

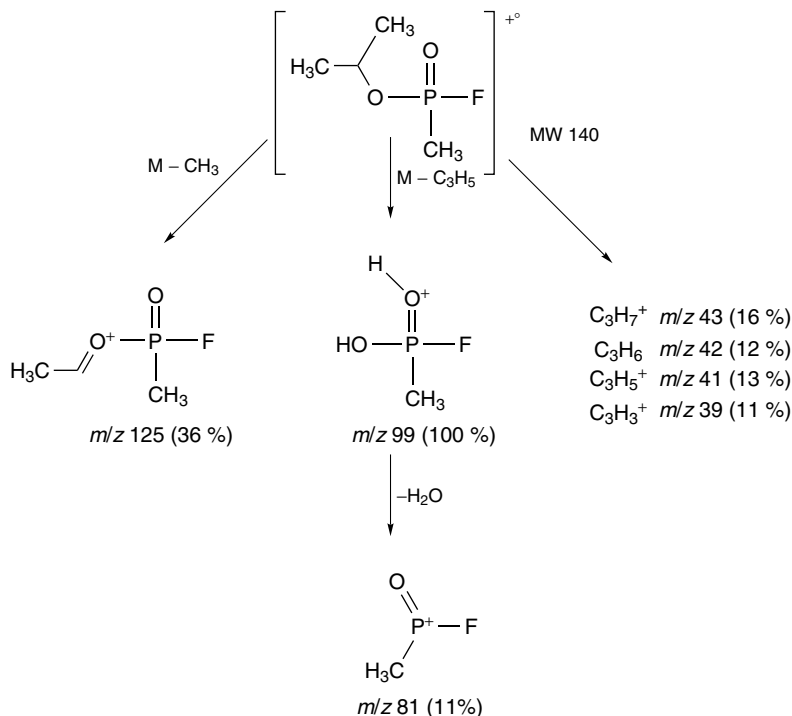
2.2 Alkyl/Cycloalkyl Alkylphosphonofluoridates

Alkyl/cycloalkyl alkylphosphonofluoridates are generally obtained from an organophosphorus precursor, typically an alkylphosphonic difluoride, and an alcohol. In 1992, over 300 aliphatic and cyclic alcohols were found to be commercially available as fine chemicals, of which inexpensive primary and secondary alcohols are considered suitable for chemical weapons production. By these criteria, approximately 70 alcohols remain of prime interest for the preparation of Schedule 1.A.1 chemicals. Only pinacolyl alcohol, the precursor of soman, is contained in the CWC Schedule

list. EI mass spectra have been recorded of the 70 corresponding alkyl/cycloalkyl methylphosphonofluoridates and for most of the corresponding alkyl (ethyl, isopropyl or propyl) phosphonofluoridates. Most of the mass spectra have been recorded as a contribution to the OPCW Analytical Database.

The EI mass spectra of the alkyl and cycloalkyl esters of methylphosphonofluoridates show particular trends. Apart from the methyl esters, all compounds eliminate an alkene/cycloalkene from the ester group. The main fragmentation of, for instance, methyl methylphosphonofluoridate (CAS 353-88-8) leads toward the base peak at mass to an electric charge ratio m/z 82, due to the loss of formaldehyde from the molecular ion (m/z 112). Methyl methylphosphonofluoridate is the only compound in the series of the recorded 70 alkyl/cycloalkyl methylphosphonofluoridates, which produces a molecular ion (int. ca. 5 %). All other compounds give rise to the phosphorus-containing ion at m/z 99 ($[\text{CH}_5\text{FO}_2\text{P}]^+$), which gives either the base peak or is highly abundant ^(2,10,17). This ion results from the loss of an alkenyl/cycloalkenyl radical from the molecular ion and is characteristic of the EI mass spectra of sarin homologues. In addition to this particular ion, the EI mass spectra show signals, due to the alkyl/cycloalkyl moiety or to phosphorus-containing fragments. Typical characteristic low mass ions are m/z 81 ($[\text{CH}_3\text{FOP}]^+$), m/z 65 ($[\text{H}_2\text{O}_2\text{P}]^+$), m/z 63 ($[\text{O}_2\text{P}]^+$), and m/z 47 ($[\text{OP}]^+$). Unfortunately, as the number of carbon atoms in the alkyl/cycloalkyl moiety exceeds six, several of the higher phosphorus-containing and hydrocarbon fragments may have the same nominal mass (e.g. m/z 99, 113, 127), and high resolution is required to separate them. As an example, the fragmentation patterns of the nerve agents sarin and soman are depicted in Schemes 1 and 2 respectively.

When comparing isomeric *O*-alkyl groups, branching has a large effect on the mass spectra ⁽¹⁷⁾. Branched chain compounds frequently display high-mass fragments in their mass spectra, due to the loss of alkyl radicals (typically: m/z 111, 125, 139, etc.) or due to the loss of alkenes (typically: m/z 112, 126, 140, etc.). This is illustrated in Figure 1 by the mass spectra of three different hexyl methylphosphonofluoridates (MW 182), of which the bottom one is from the nerve agent



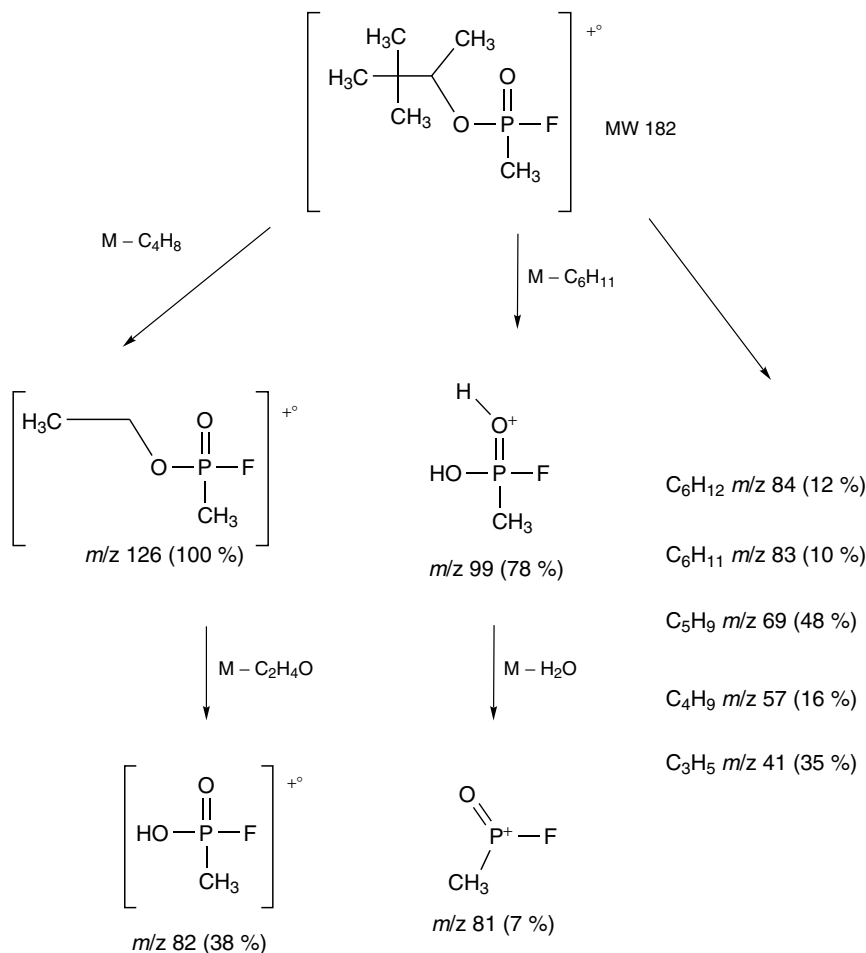
Scheme 1. Fragmentation of sarin

soman. The loss of alkene, in particular, produces information about the branching of the *O*-alkyl chain. Branching at the β -position in the *O*-alkyl chain leads to an intensive peak at m/z 112 (see Figure 1a). If the *O*-alkyl chain also contains a methyl group at position one, a strong peak at m/z 126 results (see Figure 1c). When the point of branching is further away from the phosphorus atom, the spectra (see Figure 1b) look more like the spectra of the unbranched homologues. Generally, spectra become less informative when branching is further away from phosphorus because the phosphorus-containing, high-mass fragments have a low abundance (<5%).

The high-mass ions in the EI mass spectra of the cycloalkyl homologues have generally low intensities, because fragmentation of the ester chain cannot occur easily. When the cycloalkyl ring is larger than cycloheptyl or substituted, the mass spectra correspond more to those of the corresponding cycloalkenes except from the m/z 99 peak. For an unambiguous identification by GC/MS of these

substituted cycloalkyl compounds, additional information in the form of GC retention times or retention index (RI) and CI data is essential.

The fragmentation of the other alkyl and cycloalkyl alkyl (ethyl, isopropyl, or propyl) phosphonofluoridates proceeds, in analogy to that of the sarin homologues, with the strong characteristic ions at m/z 113 (ethylphosphonofluoridates) and m/z 127 (isopropyl or propyl)phosphonofluoridates instead of m/z 99. Distinction of the propyl group linked to phosphorus can be made because the *n*-propyl isomer is capable of undergoing the McLafferty rearrangement, whereas the isopropyl is incapable of doing so. This is illustrated in Figure 2 by the mass spectra of the two isomeric sarin analogues isopropyl isopropylphosphonofluoridate (CAS 665-33-8, see Figure 2a) and isopropyl propylphosphonofluoridate (CAS 18358-37-7, see Figure 2b). The most prominent difference is the presence of m/z 98 in the mass spectrum of the propyl isomer resulting from the loss of propene from the *O*-alkyl side chain toward m/z 126 followed by the loss of ethene from the *P*-propyl moiety.



Scheme 2. Fragmentation of soman

2.3 Alkylphosphonochloridates and Alkylphosphonates Esters

Alkylphosphonochloridates may be used as starting materials for the synthesis of the corresponding alkylphosphonofluoridates. Two of these compounds, chlorosarin and chlorosoman (see Table 1), are placed on the CWC Schedule 1 list. The main peaks in the EI mass spectrum of chlorosarin (MW 156) at m/z 115/117 (loss of C_3H_5) and m/z 141 (loss of CH_3) are in accordance with the fragmentation pattern of sarin. The same analogy applies to chlorosoman (MW 198) with abundant ions at m/z 142/144 (loss of C_4H_8) and m/z 115/117 (loss of C_6H_{11}), and soman⁽¹⁰⁾.

Related to the Schedule 1 family of alkyl/cycloalkyl alkylphosphonofluoridates, are three important classes of compounds. These are the dialkyl/dicycloalkyl alkylphosphonates, the alkyl/cycloalkyl alkylphosphonates and the methyl alkyl/cycloalkyl alkylphosphonates, all belonging to the Schedule 2.B.4 chemicals. Per definition, Schedule 2.B.4 represents the largest number of chemicals of CWC interest. The first class consists of known impurities of nerve agents, the second class consists of the primarily formed hydrolysis products of nerve agents, and the third class, the corresponding methyl esters—DMMP (dimethyl methylphosphonate) and DIMP (diisopropyl methylphosphonate) (see Table 1) are

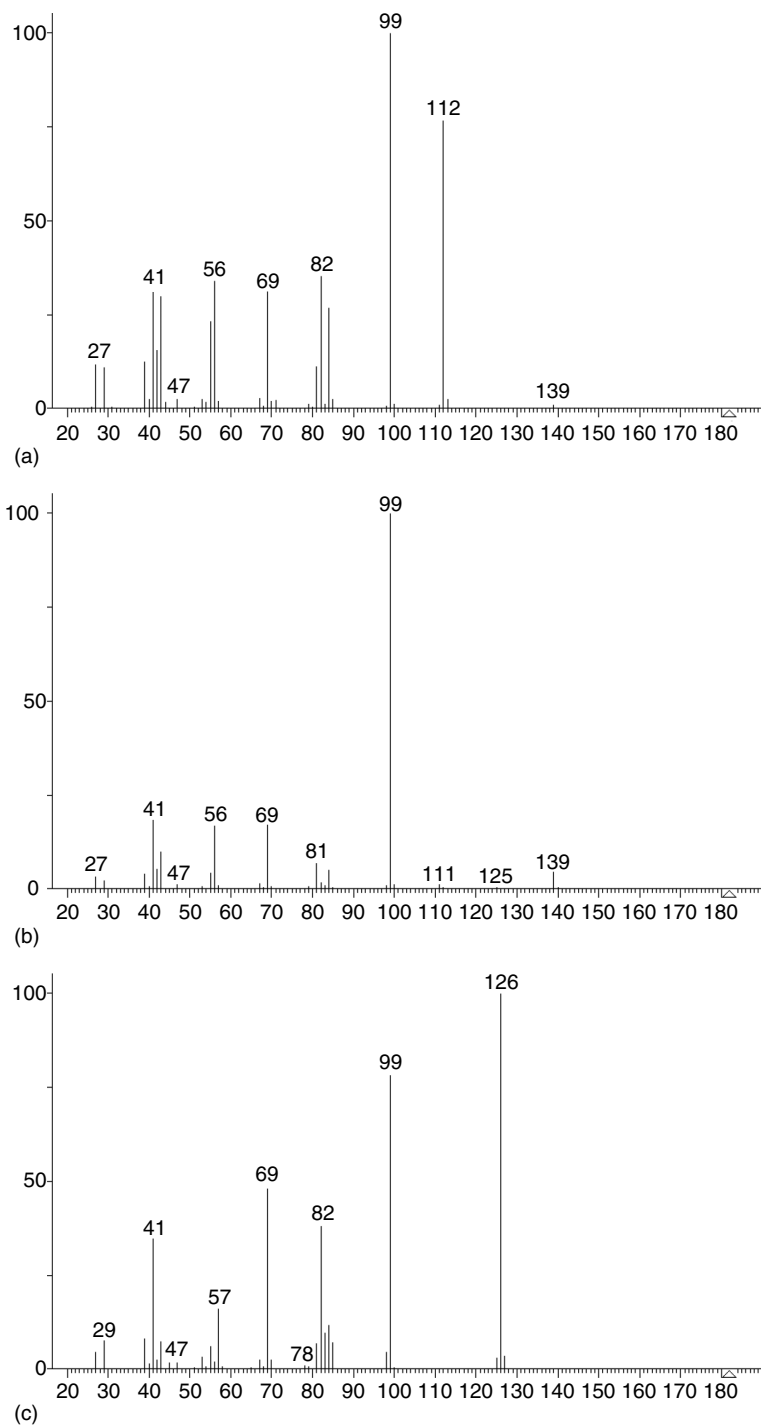


Figure 1. EI mass spectra of (a) 2-methylpentyl, (b) 4-methylpentyl, and (c) 1,2,2-trimethylpropyl methylphosphonofluoridate (soman, GD) recorded at TNO-PML (Rijswijk, The Netherlands) on a VG 70-250S GC/MS instrument (Micromass, UK)

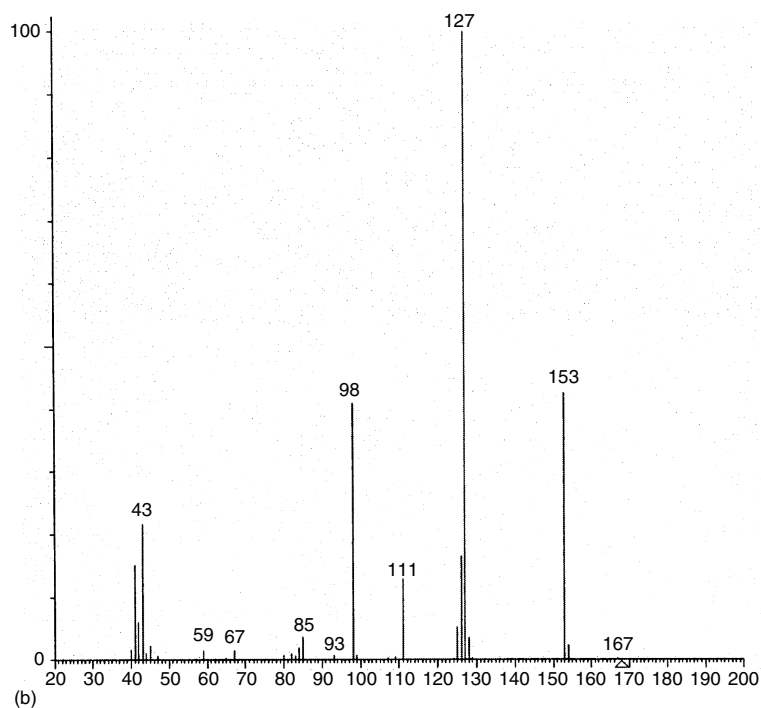
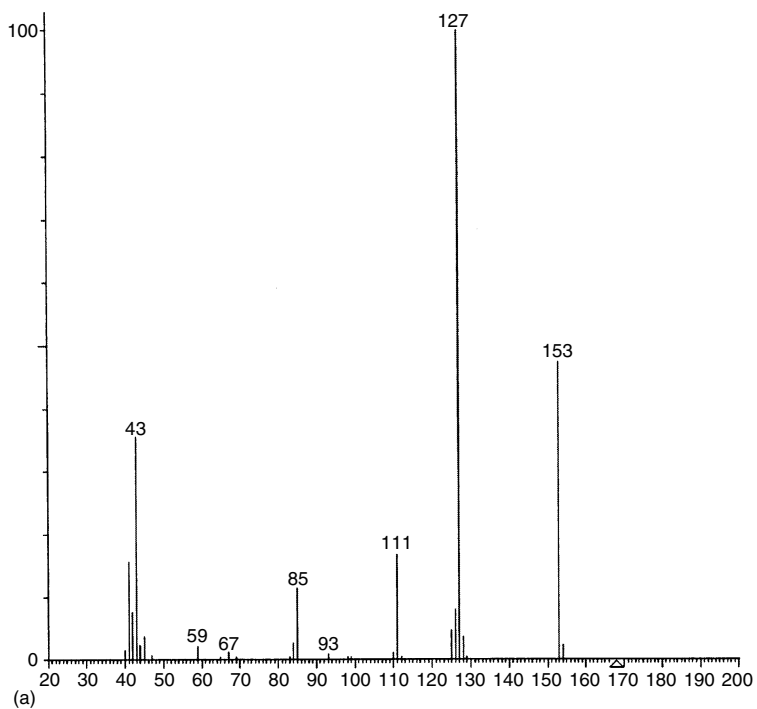


Figure 2. EI mass spectra of (a) isopropyl isopropylphosphonofluoridate and (b) isopropyl propylphosphonofluoridate recorded at the OPCW Laboratory (Rijswijk, The Netherlands) on a HP 5973 GC/MS instrument (Agilent, US)

examples of the first class of compounds and they are frequently used as simulants for nerve agents. These simple esters of methylphosphonic acid were amongst the first phosphorus compounds studied by EI/MS in the early 1960s⁽¹⁸⁾. The monoalkyl esters, also referred to as alkyl/cycloalkyl alkylphosphonic acids, are not amenable by GC/MS and require prior derivatization. EI mass spectra of the pure products can be obtained by direct inlet. The mono- and dialkyl esters, including the methyl alkyl esters, fragment by eliminating the alkyl/cycloalkyl group, analogous to alkylphosphonofluoridates. This gives rise to the following characteristic base peaks in the mass spectra:

m/z 97	(mono and di)alkyl/cycloalkyl methylphosphonates;
m/z 111	(mono and di)alkyl/cycloalkyl ethylphosphonates;
m/z 125	(mono and di)alkyl/cycloalkyl (<i>n</i> - or iso)propylphosphonates;
m/z 111	methyl alkyl/cycloalkyl methylphosphonates;
m/z 125	methyl alkyl/cycloalkyl ethylphosphonates;
m/z 139	methyl alkyl/cycloalkyl (<i>n</i> - or iso)propylphosphonates.

These base peaks are normally accompanied by abundant signals from the subsequent loss of water.

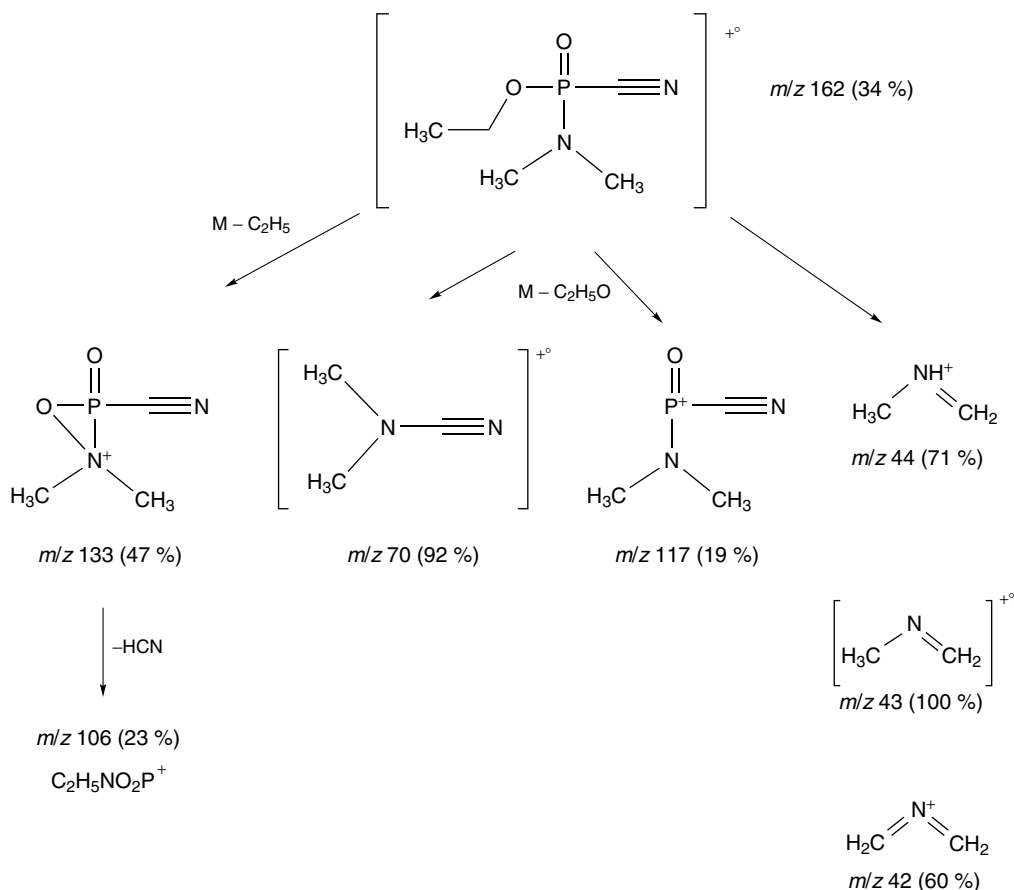
The primary elimination of an alkenyl/cycloalkenyl radical from the molecular ion in the EI mass spectra of dialkyl/dicycloalkyl alkylphosphonates generally gives rise to the most significant high-mass ion, which in turn will lose the second alkene/cycloalkene group toward the above-mentioned base peak ions. The EI mass spectrum of dicyclohexyl methylphosphonate (MW 260, CAS 7040-53-1), for example, produces a peak at m/z 179 ($[M - C_6H_9]^+$) and the base peak at m/z 97. As with the EI mass spectra of the alkylphosphonofluoridates, information on branching of the *O*-alkyl chain may be derived from other high-mass fragments.

2.4 *O*-Alkyl/Cycloalkyl *N,N*-Dialkylphosphoramidocyanidates

Tabun is the best known example of the family of *O*-alkyl/cycloalkyl *N,N*-dialkylphosphoramidocyanidates. Its fragmentation is rather different, compared

with that of the alkyl/cycloalkyl methylphosphonofluoridates. Considerable information on tabun and its impurities has become available through the GC/MS analysis of munitions-grade tabun^(13,19). The smaller phosphoramidocyanidates produce a molecular ion of moderate abundance (m/z 162 in tabun is ca. 30%). However, when the *O*-alkyl chain becomes longer, the intensity of the molecular ion signal decreases or vanishes altogether. The EI mass spectrum of tabun is dominated by the dimethylamino group (producing m/z 42, 43 and 44), and a characteristic rearrangement ion produces an intense peak at m/z 70 (see Scheme 3). The rearrangement loss of ethene or ethenyl from the ethoxy group does not occur, but the ethyl group is eliminated instead to produce the m/z 133 ion. In contrast, in the mass spectra of *O*-alkyl *N,N*-dimethylphosphoramidocyanidates with longer alkyl chains, the loss of the alkenyl radical from the molecular ion leads to a prominent ion or even to the base peak at m/z 135 ($[C_3H_8N_2O_2P]^+$). This ion is as characteristic as is m/z 99 for the alkyl/cycloalkyl methylphosphonofluoridates.

Isomerism of the *O*-alkyl chain does not influence the spectral intensities as much as with the alkyl/cycloalkyl alkylphosphonofluoridates (see Section 2.2). Therefore, a lesser number of mass spectra of compounds needs to be compiled to faithfully identify the members of the tabun family by comparison with database spectra. A series of EI mass spectra of higher *N,N*-dialkyl(ethyl, isopropyl or propyl)phosphoramidocyanidates has been recorded and contributed to the OPCW Analytical Database. In contrast to tabun, the fragmentation of the higher *N,N*-dialkyl homologues is more straightforward and proceeds in accordance with the basic rules for amines (α -cleavage) producing either abundant $[M - CH_3]^+$ or $[M - C_2H_5]^+$ ions. Subsequent elimination of the ethene and/or propene and HCN take place, leading to the most abundant peaks in the mass spectra. This is illustrated in the mass spectra of the three tabun homologues *O*-ethyl *N,N*-diethylphosphoramidocyanidate (MW 190, CAS 63815-60-1, see Figure 3a), *O*-ethyl *N,N*-diisopropylphosphoramidocyanidate (MW 218, see Figure 3b), and *O*-ethyl *N,N*-dipropylphosphoramidocyanidate (MW 218, see Figure 3c).



Scheme 3. Fragmentation of tabun

2.5 *O*-Alkyl/Cycloalkyl *S*-2-Dialkylaminoethyl Alkylphosphonothiolates

The EI mass spectra of the *O*-alkyl/cycloalkyl *S*-2-dialkylaminoethyl alkylphosphonothiolates are dominated by the *S*-2-dialkyl(methyl, ethyl, isopropyl, propyl)aminoethyl moieties. This leads for VX to extensive ions at m/z 127, 114, 72, and 30 (see Scheme 4). As for tabun, the GC/MS analysis of munitions-grade VX provided a valid source of information⁽³⁾. The phosphorus-containing fragments are characteristic (m/z 252, 167, 139), but low in abundance. In the mass spectra of the *S*-2-diethylaminoethyl (producing m/z 99, 86, 58) and *S*-2-dimethylaminoethyl (producing m/z 71, 58) homologues, corresponding fragmentations occur.

In an analogous manner to the behavior of the tabun family, branching of the alkyl/cycloalkyl chain does not have a large influence, and mass spectra of a few compounds are generally sufficient for the primary identification of VX family members. A series of EI mass spectra of *O*-alkyl/cycloalkyl *S*-2-dialkylaminoethyl alkyl(ethyl, isopropyl, or propyl)phosphonothiolates has been recorded and contributed to the OPCW Analytical Database.

2.6 Vesicants

EI mass spectra of mustard gas (see Table 1) and several derivatives, including its main precursor and hydrolysis product thiodiglycol, have been described^(9,11). The fragmentation of mustard gas

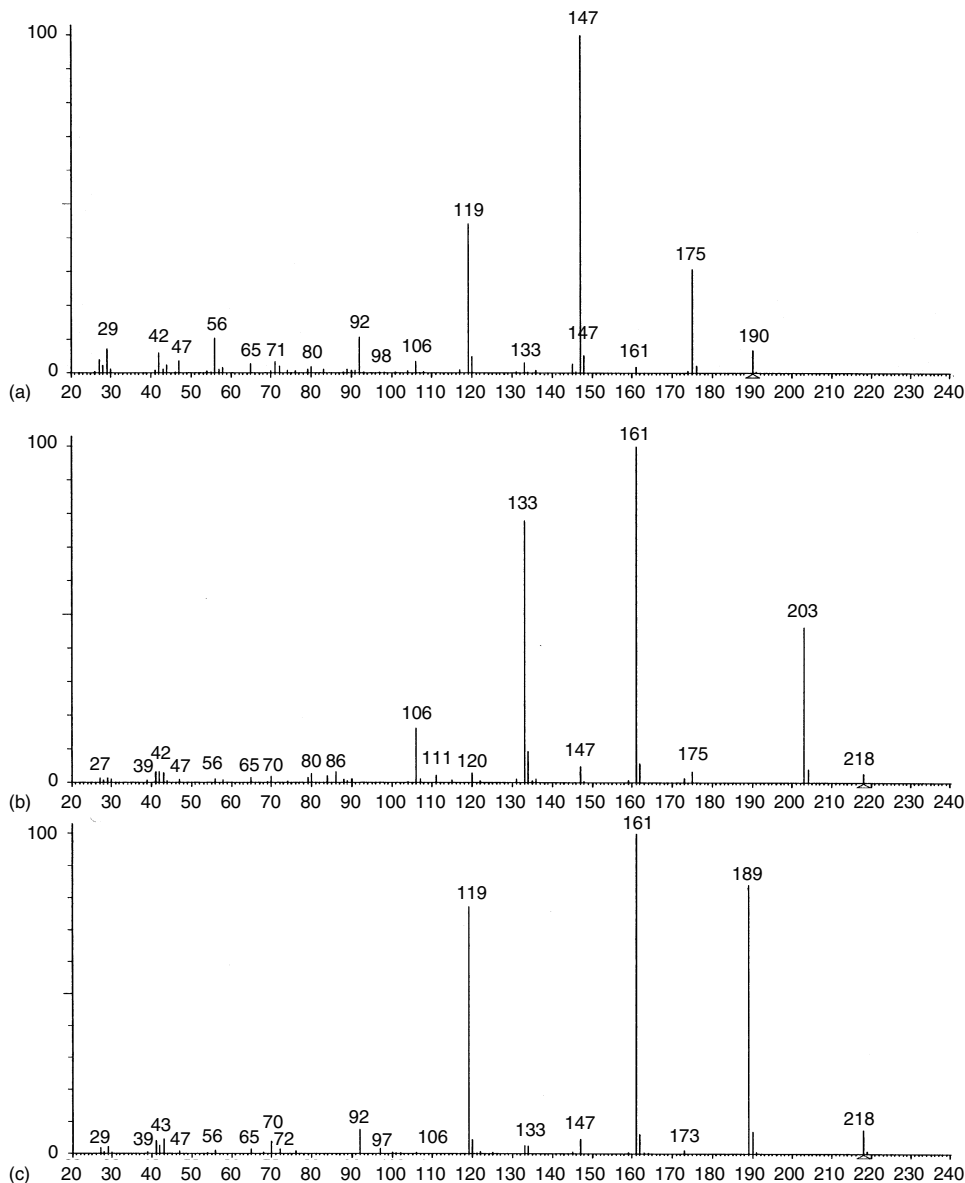
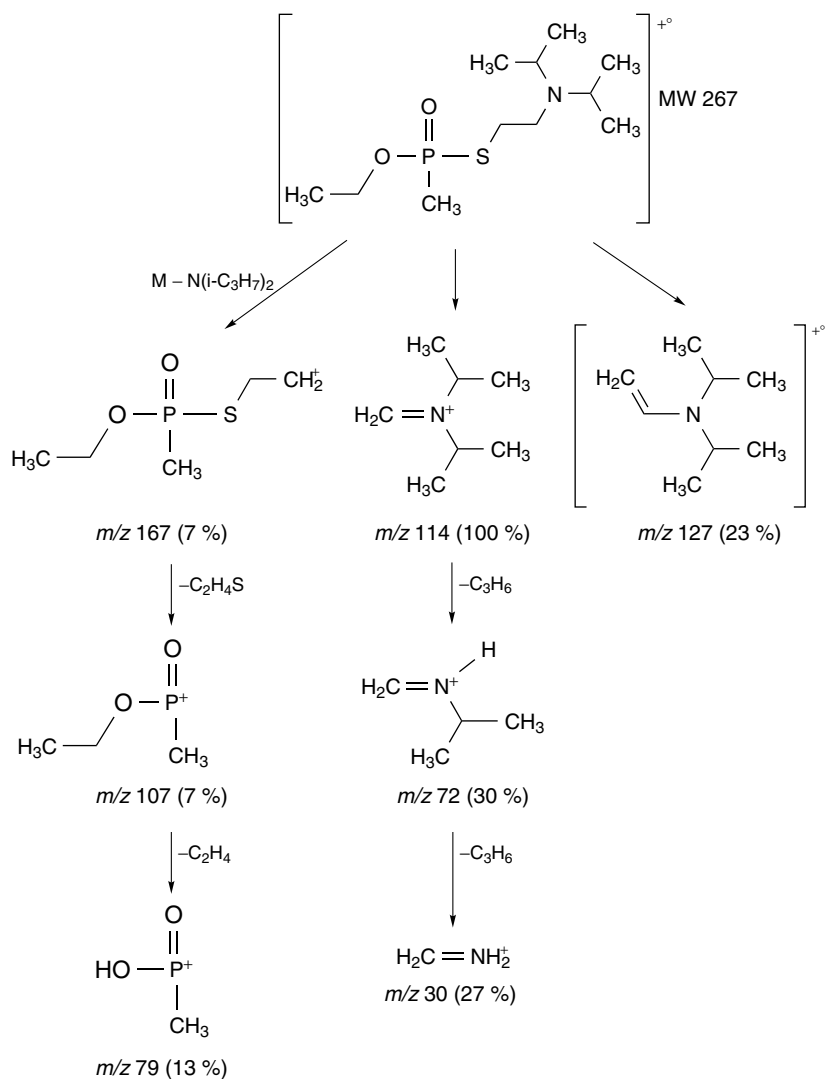
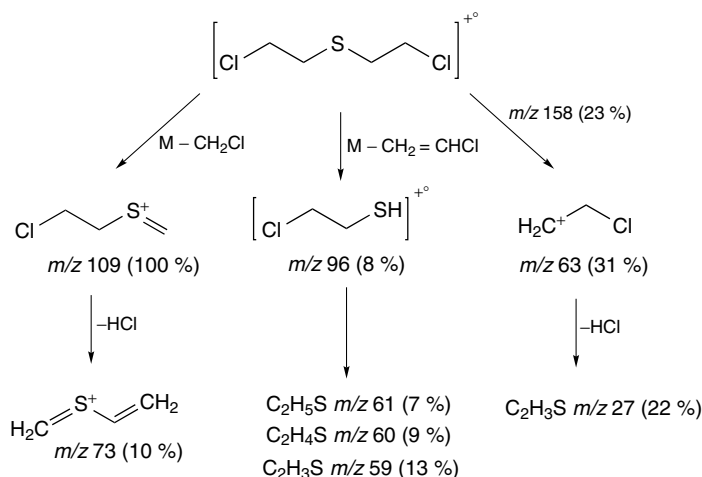


Figure 3. EI mass spectra of (a) *O*-ethyl *N,N*-diethylphosphoramidocyanidate, (b) *O*-ethyl *N,N*-diisopropylphosphoramidocyanidate, and (c) *O*-ethyl *N,N*-dipropylphosphoramidocyanidate recorded at the Military Technical Institute of Protection (Brno, The Czech Republic) on a HP 5971A GC/MS instrument (Agilent, US)

follows the same pattern as with other dialkyl sulfides. Cleavage of the bond β to the sulfur with charge retention at that atom (thioether cleavage) and cleavage of the C–S bond with charge retention at the chloroalkyl fragment are two major fragmentation routes. With mustard gas (MW 158),

this gives rise to the base peak at m/z 109/111 and an intensive signal at m/z 63/65 (see Scheme 5). MW information can be obtained for mustard gas and sesquimustard (see Table 1, MW 218). However, with an increase of the length in the series of mustard gas homologues, the intensity of the molecular ion

**Scheme 4.** Fragmentation of VX



Scheme 5. Fragmentation of mustard gas

decreases. Cleavage of the C–S bond with elimination of the S-CH₂CH₂Cl moiety produces the base peak at m/z 123/125 in the EI mass spectrum of sesquimustard. Other strong peaks are at m/z 63/65 and m/z 109/111. Considerable information on mustard gas decomposition products and impurities in technical mustard gas has become available through the GC/MS analysis of a block of mustard gas fished up from the Baltic Sea ⁽²⁰⁾. Over 30 compounds were identified, of which most of them are not covered by the CWC Schedule list. Mass spectra of mustard gas related vesicants listed in the CWC Schedules are taken up in the OPCW Analytical Database.

The fragmentation of lewisite 1 (see Table 1) is rather straightforward ⁽⁹⁾. A strong molecular ion cluster at m/z 206 is formed under EI conditions and prominent peaks at high-mass values are observed, including a rearrangement toward arsenic trichloride at m/z 180/182/184 and m/z 145/147 (see Scheme 6). The *cis/trans* forms of lewisite 1 have identical EI mass spectra. A technical lewisite (L) consists mainly of the *trans* isomer. The EI mass spectra of L 2 and L 3 (see Table 1) have also been measured. Both compounds produce molecular ion signals (L 2 at m/z 232, 234, and 236 and L 3 at m/z 258, 260, and 262) and fragment in an analogous manner to lewisite 1.

The fragmentation of the three nitrogen mustards (HN-1 (ethyl nitrogen mustard), HN-2 (methyl nitrogen mustard), and HN-3 (nitrogen mustard))

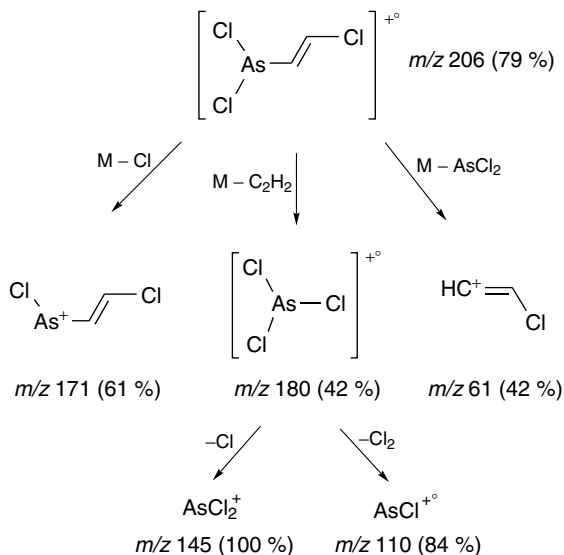
proceeds in accordance with the basic fragmentation rules for amines (α -cleavage). This results in EI mass spectra with the following base peaks:

- m/z 106/108 for HN-2 (Cl₁, see Table 1, MW 155);
- m/z 120/122 for HN-1 (Cl₁, see Table 1, MW 169);
- m/z 154/156/158 for HN-3 (Cl₂, see Table 1, MW 203).

The molecular ion signals in the mass spectra of the nitrogen mustards are weak (<5%), and the peak originating from the 2-chloroethyl group (m/z 63/65) is rather prominent (>20%).

2.7 Other Chemical Weapons Convention Scheduled Compounds

In addition to chlorosarin and chlorosoman (see Section 2.3), there are two families of key precursors placed on the Schedule 1 list. DF (see Table 1) is the best known example of the Schedule 1.B.9 chemicals. The molecular ion, at m/z 100, gives the base peak in its EI mass spectrum ⁽⁴⁾. The fragmentation is somewhat complicated, but gives abundant peaks for the loss of CH₃O (m/z 69) and HF (m/z 80). The trivalent alkyl/cycloalkyl 2-dialkylaminoethyl alkylphosphonites (Schedule 1.B.10), which are related to the VX family of compounds, have QL (see Table 1) as their main representative because



Scheme 6. Fragmentation of lewisite 1

this is the key precursor for the production of VX by a binary process. All main fragments in the mass spectrum of QL can be attributed to the diisopropylaminoethyl group, and none of the eight strongest peaks are phosphorus-containing fragments ⁽⁴⁾.

In contrast to the vast number of Schedule 1.A chemicals, there are only three toxic chemicals placed under Schedule 2.A of the CWC Schedule list, namely, Amiton, PFIB, and BZ (see Table 1). Amiton (MW 269) produces an EI fragmentation pattern analogous to that of the structurally related VX, with strong peaks originating from the *S*-2-diethylaminoalkyl moiety (*m/z* 99, 86, 58). The molecular ion signal at *m/z* 269 is absent; the highest peak of any significance is at *m/z* 197 (ca. 1–2 %). PFIB (MW 200) produces a molecular ion and peaks typical of fluorine-containing ions (*m/z* 181, base peak; *m/z* 69, $[\text{CF}_3]^+$). The compound is extremely volatile and requires special GC conditions (subambient temperature; special columns) in order to be analyzed by GC/MS. The EI mass spectrum of BZ has been studied along the spectra of a series of other psychoactive glycolates ⁽¹²⁾. The spectrum displays a weak molecular ion signal at *m/z* 337, and strong peaks arising from the diphenylglycolic (*m/z* 183 and 105) and from the quinuclidinyl part (*m/z* 126) of the molecule. The same

strong peaks at *m/z* 105 (benzoyl cation) and *m/z* 183 (loss of COOH) are found in the EI mass spectrum of benzoic acid (MW 228, see Table 1), the key precursor for the production of BZ ⁽⁴⁾. Peaks from the second BZ precursor 3-quinuclidinol (MW 127, see Table 1) are observed in the EI mass spectrum of BZ. Although BZ and 3-quinuclidinol can be determined as such by GC/MS, the GC performance is improved significantly after derivatization of the hydroxy group. Benzoic acid cannot be determined as such by GC/MS and conversion into a volatile nonpolar analytical derivative is required.

The Schedule 2 list contains two classes of precursors for the production of tabun and its homologues. *N,N*-dimethylphosphoramidic dichloride (see Table 1) can be converted to tabun with ethanol and a cyanide. Its EI mass spectrum is rather straightforward, with signals due to the molecular ion cluster (*m/z* 161/163/165), to the loss of a hydrogen (*m/z* 160/162/164), and a chlorine radical (*m/z* 126/128), and to the dimethylamino group (*m/z* 42, 43, 44). Diethyl *N,N*-dimethylphosphoramidate (see Table 1) is the best known example of the second class of possible tabun precursors. The compound was also detected as an impurity in munitions-grade tabun ⁽¹³⁾. The molecular ion (*m/z* 181, 15 %) fragments by losing an ethyl radical

(to m/z 162), in analogy to tabun, and subsequent elimination of ethene (to m/z 124). The spectrum base peak, at m/z 44, is due to the dimethylamino group.

The Schedule 2 list contains three classes of dialkyl(methyl, ethyl, propyl, and isopropyl)aminoethane compounds, namely, 2-chlorides, 2-ols, and 2-thiols. All three classes are possible precursors of VX and its homologues. In an analogous manner to other simple scheduled chemicals, EI mass spectra are found in commercially available mass spectral databases. Table 1 contains information on the diisopropylaminoethyl compounds. The EI mass spectra of the alcohol (MW 145) and the thiol (MW 161) show the α -cleavage signal, at m/z 114 (base peak), and typical signals for the subsequent elimination of alkenes, at m/z 72 and m/z 30. In the EI mass spectra of the chloride (MW 163), cleavage preferably takes place at the *N*-isopropyl group, giving m/z 148, and is followed by a loss of propene, resulting in the base peak at m/z 106.

In addition to the dialkylaminoethane compounds, organophosphorus precursors are needed for the production of Schedule 1.A.3 chemicals. These precursors, such as methylthiophosphonic dichloride (see Table 1), are not specifically mentioned in the Convention text, but they belong to Schedule 2.B.4. The EI mass spectra of the closely related and important precursors, methylphosphonic dichloride (MW 132) and methylthiophosphonic dichloride (MW 148), are dominated by the molecular ion cluster and display the base peak signal for $[M - Cl]^+$. The relatively stronger molecular ion signal in the mass spectrum of the thio compound reflects the larger stability of the ionized molecule. The higher molecular ion signal in EI mass spectra of organothiophosphorus compounds, as compared to the corresponding mass spectra of their oxygen analogues, is a general feature⁽¹⁵⁾.

The Schedule 3 list contains only 17 discrete chemicals, of which four represent toxic chemicals. One of them is too simple (HCN) to produce an informative mass spectrum and some of the precursors (chlorinating chemicals) cannot be analyzed by GC/MS. Mass spectra are contained in the OPCW Analytical Database and in commercially available databases. The mass spectrum of trichloronitromethane (chloropicrin, CAS 76-06-2) is almost identical to that of trichloromethane (chloroform), apart from a peak at m/z 30. Not scanning this low

mass ion may lead to a false-negative identification. The phosphites were among the first phosphorus compounds studied by EI/MS in the early 1960s⁽¹⁷⁾. The three ethanolamines, used for the production of nitrogen mustards, need derivatization before proper GC/MS analysis.

2.8 Reproducibility of Mass Spectra

Reproducibility of the relative intensities in EI mass spectra is a concern, despite the fact that spectra are recorded under standardized conditions. Different mass spectrometers produce spectra with, sometimes, large spreads in the relative intensities. An impression of the possible variation is given in Table 2. In this table, the relative intensities of major fragments in the EI mass spectra of three nerve agents, taken from three different sources, are presented. It is clear that there exist no reference mass spectra of these Schedule 1 chemicals, which can be considered as true physical constants. A compilation of more than one spectrum of the same compound in the OPCW Analytical Database gives an indication of the possible spread.

3 CHEMICAL IONIZATION MASS SPECTROMETRY OF CHEMICAL WEAPONS CONVENTION SCHEDULED COMPOUNDS

Although EI is the most widely used ionization method, there is a clear need for CI data for identifying CWC-related chemicals by GC/MS. Extensive fragmentation with resultant loss of MW information is particularly observed in EI mass spectra of the organophosphorus compounds of Schedules 1.A.1 (sarin family) and 1.A.3 (VX family). As an example, Figure 4(a) shows the EI mass spectrum of the nerve agent VX. The molecular ion at m/z 267 is absent. Although the pattern of the peaks is characteristic, complete identification is only possible after observation of the pseudomolecular ion at m/z 268 by CI (see Figure 4(b)). Fragmentation of VX takes place under ammonia CI conditions, but by a different mechanism when compared to EI/MS. By using

Table 2. Major fragments in the EI mass spectra of three nerve agents taken from three different sources

Sarin				Soman				Tabun			
<i>m/z</i>	int. (%) ^a	int. (%) ^b	int. (%) ^c	<i>m/z</i>	int. (%) ^a	int. (%) ^b	int. (%) ^c	<i>m/z</i>	int. (%) ^a	int. (%) ^b	int. (%) ^c
41	12	7	13	41	18	37	35	42	29	43	60
42	10	5	12	43	10	15	8	43	72	100	100
43	20	8	16	82	40	50	38	44	43	55	71
79	10	2	3	84	25	10	12	70	100	85	92
81	20	10	11	99	100	85	78	106	29	21	23
99	100	100	100	126	88	100	100	117	29	14	19
125	25	33	36	127	5	4	4	133	43	42	47
								162	32	29	34

^aRecorded on a Hewlett-Packard Dodecapol Model 5982A (USA), 70 eV, 150 °C source temperature ⁽²⁾. (Reproduced by permission from Sass, S. and Fisher, T. L., *Org. Mass Spectrom.*, 14, 257–264 (1979), Copyright John Wiley & Sons, Ltd.) ^bRecorded on a Jeol JMS-01-SG-2 magnetic sector instrument (Japan), 75 eV, 260 °C source temperature ⁽⁵⁾. (Reproduced by permission of VERIFIN from 'Identification of Potential Organophosphorus Warfare Agents', Ministry for Foreign Affairs of Finland, Helsinki, 1979.) ^cRecorded at TNO-PML on a Micromass VG 70-250S magnetic sector instrument (UK), 70 eV, 200 °C source temperature.

methanol as CI gas, the protonated molecular ion turned out to be the base peak in the mass spectrum of VX and several of its degradation products ⁽²¹⁾.

Several investigations have been performed on the use of CI in the field of CW agents and related compounds, and the influence of the various reagent gases on the selectivity and sensitivity of GC/CI/MS has been described ^(2,21–24). Methane, isobutane, ammonia, ethylene, and methanol have been applied in the formation of CI reagent ions. The first three gases are commonly used for GC/CI/MS of scheduled chemicals and methane CI mass spectra of nerve agents were published in 1979 ⁽²⁾. Among these three gases, ammonia has the lower proton affinity (PA) and methane has the highest, whereas isobutene (neutral formed from isobutane reactant ions) has an intermediate PA. Therefore, CI with methane generally results in more extensive fragmentation than CI with isobutane or ammonia. In addition, ammonia often forms adduct ions, $[M + \text{NH}_4]^+$, with many compounds. The ion source temperature may have an effect on the intensity of the pseudomolecular ions and lower source temperatures are generally favored. The information content of CI mass spectra is typically limited to MW information, which is easily accessible from simple calculations. In addition, CI mass spectra vary strongly with CI conditions (choice of gas, etc.) and are more strongly influenced by the instrument used than EI mass spectra. Therefore, compilations of CI/MS data are not as widely used as EI mass spectral databases.

Ammonia CI proved to be very useful for the organophosphorus CW agents leading to abundant $[M + \text{H}]^+$ and/or $[M + \text{NH}_4]^+$ pseudomolecular ions ⁽²²⁾. Owing to their relative low PA, ubiquitously present hydrocarbons will not be ionized, opening up the possibility for more selective detection of other compounds. The difference between using isobutane or ammonia CI for mustard gas and related compounds has been investigated ^(23,24). Both gases provide MW information in the form of either $[M + \text{H}]^+$ or $[M + \text{NH}_4]^+$ peaks, but isobutane lacks the selectivity of ammonia. This was demonstrated by the detection of the mustard gas impurity 2-chloroethyl (2-chloroethoxy)ethyl sulfide (CAS 114811-38-0) under ammonia CI conditions in the presence of co-eluting hydrocarbons, achieved by ammonia CI but impossible by isobutane CI ⁽²⁴⁾.

The relatively soft ionization conditions of isobutane and ammonia CI often prevent the observation of fragment ions for identification purposes. This lack of information can be overcome by the use of tandem mass spectrometry (MS/MS) analysis, in which fragmentation can be obtained by collision induced dissociation (CID) of the pseudomolecular ions. Scheduled chemicals have mostly been investigated under positive ion CI conditions, but negative ion CI can be used in principle. However, of the compounds described in Section 2, only PFIB generates negative ions by electron capture; in this particular case, the negative ion CI detection limit lies two orders of magnitude below that observed with EI.

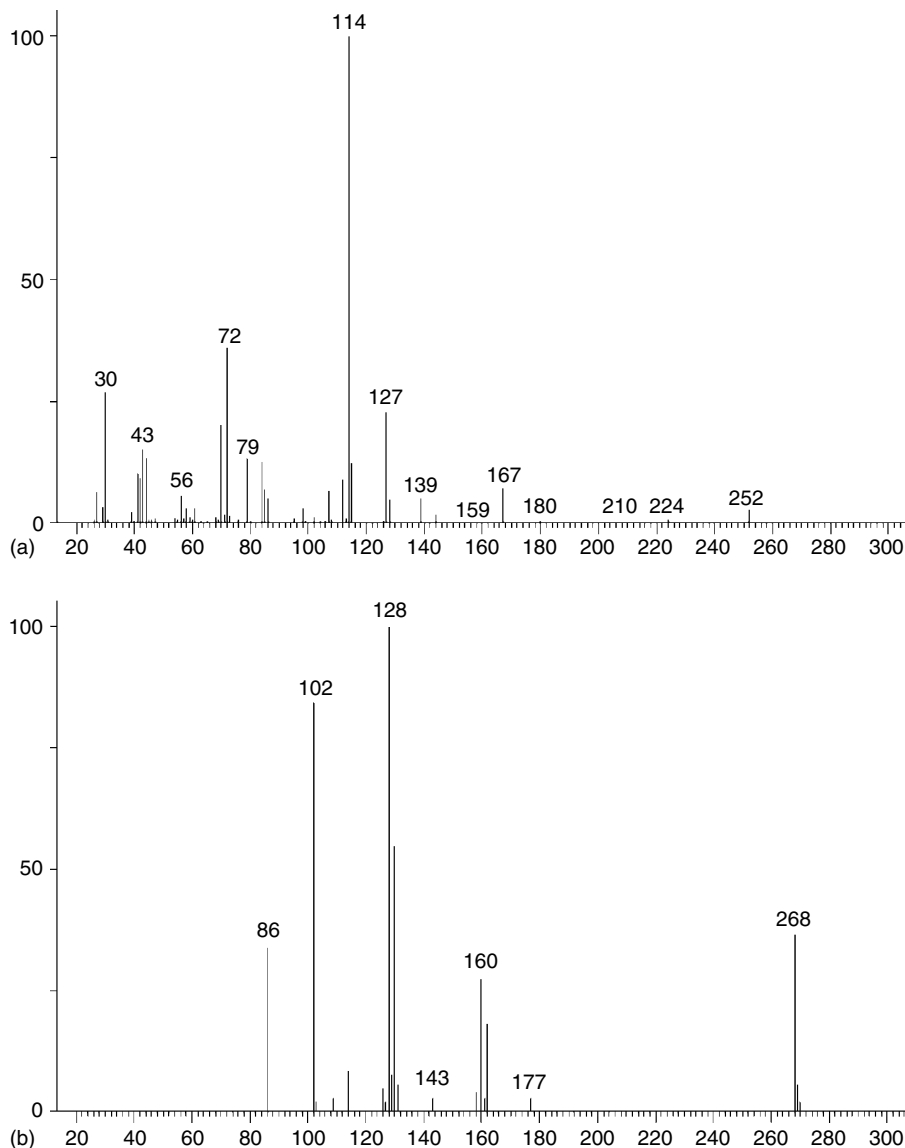


Figure 4. EI (a) and ammonia CI (b) mass spectra of VX recorded at TNO-PML on a VG 70-250S GC/MS instrument (Micromass, UK)

4 SPECIAL GAS CHROMATOGRAPHY/MASS SPECTROMETRIC TECHNIQUES

In cases where low detection limits are required, single ion monitoring (SIM) or multiple ion monitoring (MIM) can be applied. Although less specific

than full scan MS, a factor of 100–1000 times greater sensitivity may be reached, to allow the detection of scheduled chemicals at low picogram levels per injection. However, for scheduled chemicals with a strong adsorptive nature, such as the nerve agent VX, this low level of detection may not be feasible. The selection of ion masses for

SIM or MIM will depend on the compound and the background of the sample matrix, as well as on the GC column, but in general, high and even masses will be more specific. This may not be the case for the alkyl/cycloalkyl alkylphosphonofluoridates, as the masses (e.g. m/z 99, 113, 127) arising from the organophosphorus moiety under EI conditions coincide with those of ubiquitously present hydrocarbons. In order to be useful for identification, GC/MS(MIM) has to be performed on at least three individual ions of a significant mass spectral ($>10\%$ of the base peak) intensity. The GC peaks obtained during the analysis must have coincident maxima, the same peak-width-at-half-height, and a signal-to-noise ratio exceeding three. Furthermore, the retention time must agree with that of an authentic reference compound, and the intensity ratio of the selected ion signals should fall within 10% of that of the reference compound. The comparison of the retention time and spectral intensity ratio should be conducted with the same equipment and under the same experimental conditions.

By applying high-resolution GC/MS with a magnetic sector mass spectrometer, the exact mass of a fragment can be measured, allowing the calculation of the corresponding elemental composition. This provides a valuable tool for the identification of unknowns. Some fragments of Schedule 1 chemicals are extremely specific, for example, $[\text{CH}_5\text{FO}_2\text{P}]^+$ (m/z 99.0011) in the spectra of the sarin homologues. This ion can easily be distinguished from a possible interfering hydrocarbon background ion $[\text{C}_7\text{H}_{15}]^+$ (m/z 99.1174), even by the moderate resolution of 5000 (10% valley). High-resolution SIM improves the selectivity and may even allow a higher sensitivity as compared with low-resolution SIM, despite the fact that the ion throughput of the instrument is reduced at higher resolution. Detection levels of even less than 1 pg per injection can be reached under optimal conditions.

GC/MS/MS also allows the determination of scheduled chemicals in complex sample matrices with great selectivity and at low detection levels. Information by MS/MS can be obtained either by recording product (parent–daughter) ion spectra of a selected mass or by performing single reaction monitoring (SRM) or multiple reaction monitoring

(MRM). In the latter case, a specific fragmentation is monitored, for example, by selecting a specific parent ion mass in the first mass analysis step, and by transmitting one (SRM) or a few (MRM) formed fragments in the second mass analysis step. Compared to SIM, the selectivity is greatly enhanced. Several applications of this method have been described in the field of CWC-related chemicals, for instance, the determination of small quantities of the nerve agents sarin and soman in air, contaminated with a huge amount of diesel fuel and sampled on a respirator canister. Reported detection limits for GC/CI/MS/MS(SRM) of ammonium adduct ions were 15 pg for sarin (transition m/z 158 to m/z 99) and 80 pg for soman (transition m/z 200 to m/z 183) per injection ⁽²⁵⁾. A similar GC/tandem MS approach was applied for the detection, under both EI and CI conditions, of several alkyl methylphosphonates in complex mixtures after converting the acids to their trimethylsilyl esters ⁽²⁶⁾. This shows that the extra dimension of MS/MS adds to the power of GC/MS in the analysis of complex mixtures.

The application of several of the above described GC/MS techniques to real-world samples (clothing, grave debris, soil, and munitions fragments) collected from a Kurdish village in northern Iraq after alleged CW agents attacks in 1992 demonstrates that unequivocal identification can be carried out on different samples with analyte concentrations ranging from a low microgram per kilogram to milligram per kilogram level ⁽²⁷⁾. As an illustrative example, the result of a GC/MS/MS(MRM) analysis of a metal fragment extracted with dichloromethane is depicted in Figure 5. The transition monitored was m/z 125 to m/z 99 and 81 proving the presence of the nerve agent sarin.

5 ANALYTICAL DERIVATIZATION FOR GAS CHROMATOGRAPHY/MASS SPECTROMETRIC ANALYSIS

Derivatization of analytes may be necessary in order to make them amenable to GC/MS analysis. In most cases of verification analysis, derivatization involves the conversion of polar, nonvolatile degradation products of scheduled chemicals into more

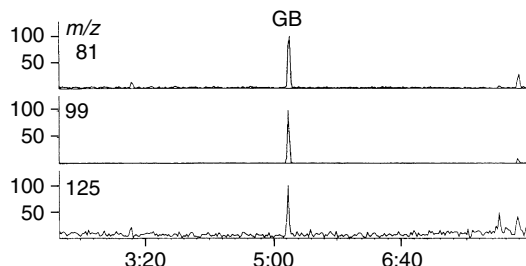


Figure 5. Result of the GC/MS/MS(MRM) analysis of a metal fragment extracted with dichloromethane proving the presence of sarin. (a) m/z 125 \rightarrow m/z 81, (b) m/z 125 \rightarrow m/z 99, and (c) m/z 125 (unfragmented). Data recorded at DSTL, Porton Down (UK) on a Finnigan MAT TSQ 700 GC/MS instrument (Finnigan MAT, UK) ⁽²⁷⁾. (Reproduced from Black, R. M., Clarke, R. J., Read, R. W. and Reid, M. T. J., *J. Chromatogr., A*, 662, 301–331 (1994) by permission of Elsevier Science)

volatile nonpolar compounds. Although the direct analysis of polar compounds by LC/MS is more straightforward, this analysis is often confirmed by the GC/MS analysis of the derivatized compounds. As polar compounds are normally in an aqueous matrix, this requires the removal of water. This can be accomplished by evaporation to dryness and redissolution of the residue in a suitable organic solvent. Care should be taken that polar but nonionic analytes, such as thiodiglycol, do not evaporate. A problem may occur with divalent organophosphorus acids because they form insoluble salts with metal ions (e.g. Na^+ , K^+ , Mg^{2+} , Ca^{2+}) present in environmental samples. Removal of the cations with a cation-exchange cartridge before the evaporation to dryness may solve this problem. Contamination of the GC/MS system (injection system, column) may occur, especially with divalent acids such as methylphosphonic acid, in cases where derivatization is incomplete.

There are several derivatization reagents. The following general reagents are the most frequently used in verification analysis:

- Diazomethane: this reagent converts acids into methyl esters and is used for the conversion of alkylphosphonic acids into methyl alkylphosphonates. It is one of the most potent methylation reagents. However, it does not methylate the polar alcohols of the CWC Schedule list, for example, thiodiglycol.
- Bis(trimethylsilyl)trifluoroacetamide (BSTFA): among the many commercially available trimethylsilylation reagents, BSTFA is a popular one.

It converts acids into trimethylsilyl (TMS) esters and polar alcohols into TMS ethers. However, the TMS derivatives are easily hydrolyzed by traces of water, and measurements should proceed directly after derivatization.

- *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA): this reagent has several advantages above its corresponding TMS analogue, BSTFA. The derivatization occurs already at room temperature, and the derivatives obtained are more stable toward hydrolysis ⁽²⁸⁾.

Methyl esters of alkylphosphonic acids may also be obtained by heating these acids together with trimethylphenylammonium hydroxide in the hot (e.g. 250 °C) injection port of the GC/MS system.

As an example, EI mass spectra of the different derivatives of isopropyl methylphosphonate (CAS 1832-54-8), the primary hydrolysis product of the nerve agent sarin, are presented in Figure 6. The EI mass spectra of the methyl alkylphosphonates produce rather characteristic organophosphorus-containing fragments (see Section 2.3), which is an advantage over the silylated alkylphosphonates. In contrast, the behavior of ionized silylated alkylphosphonates is dominated by the TMS or *tert*-butyldimethylsilyl (TBDMS) moieties ⁽²⁸⁾. An ion at high mass, $[\text{M} - \text{CH}_3]^+$ and $[\text{M} - \text{C}_4\text{H}_9]^+$, is usually observed in the EI mass spectra of the TMS and TBDMS esters respectively. Both esters produce the same base peak, at m/z 153, which reflects the elimination of the alkene group (see Figure 6). The base peak or the $[\text{M} - \text{CH}_3]^+ / [\text{M} - \text{C}_4\text{H}_9]^+$ ion could be selected for SIM or daughter scan by

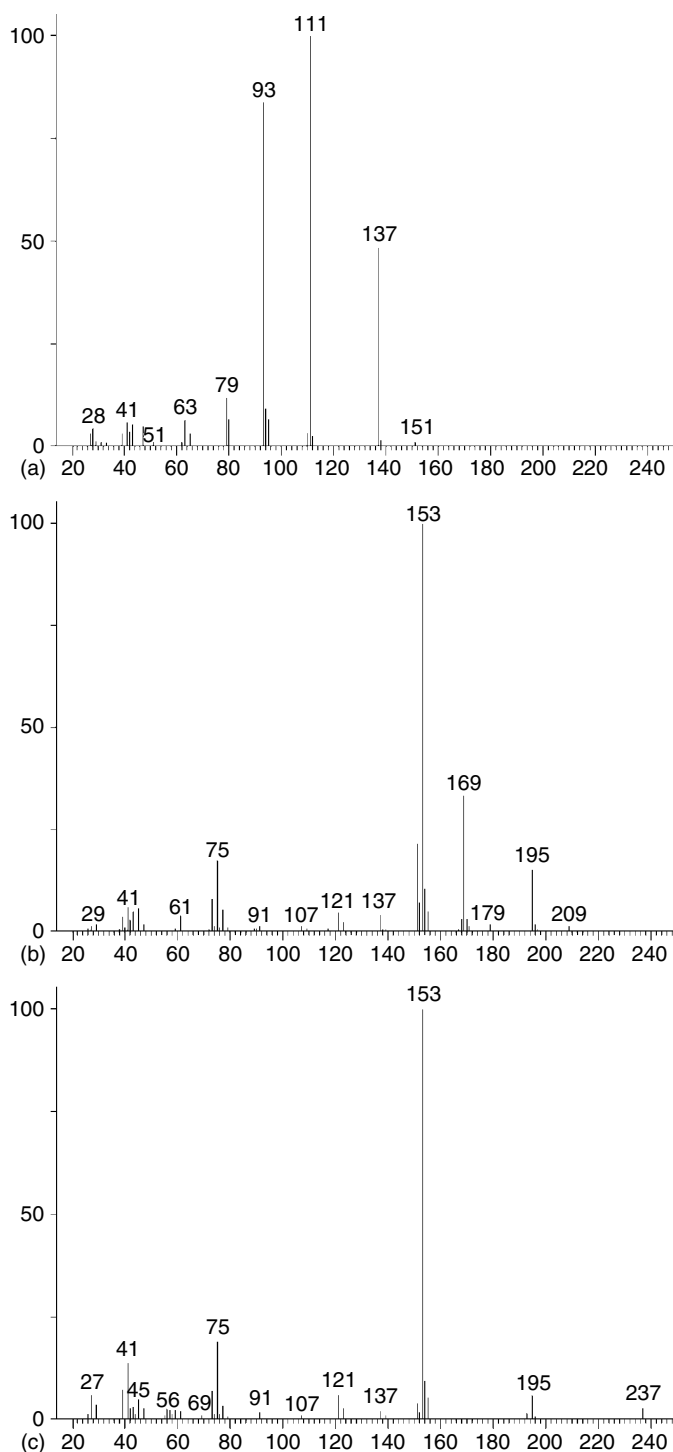
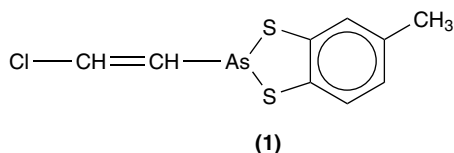


Figure 6. EI mass spectra of (a) isopropyl methyl methylphosphonate (MW 152, CAS 690-64-2), (b) isopropyl TMS methylphosphonate (MW 210), and (c) isopropyl TBDMS methylphosphonate (MW 252, CAS 126281-76-3) recorded at TNO-PML on a VG 70-250S GC/MS instrument (Micromass, UK)

GC/MS/MS. The three derivatization reactions are routinely carried out in most laboratories, with a slight privilege for trimethylsilylation. This preference is reflected in the fact that trimethylsilylation was selected for the OPCW on-site sample preparation methods for GC/MS. However, when unknown samples have to be analyzed both methylation and silylation are recommended.

In addition to an increase in the volatility of the analytes, derivatization may also bring about an enhancement in the sensitivity of detection. This is particularly relevant with fluorine-containing reagents (perfluorobenzylation, trifluoroacetylation, heptafluorobutylation), followed by GC/MS analysis under electron-capture negative-ion CI conditions.

A special derivatization reaction is required for lewisite 1, which is so reactive that it cannot be determined by GC/MS in low quantities (e.g. below 10 ng per injection). It has been known for a long time that lewisite 1 reacts with compounds having an α, β -dithiol structure, such as 2,3-dimercaptopropanol-1 (British-Anti-Lewisite (BAL); also used for medical treatment). The derivatization reaction can be performed at an analytical level and several examples have been described⁽²⁹⁾. The reaction product of lewisite 1 with 3,4-dimercaptotoluene, 2-(2-chlorovinyl)-5-methyl-1,3,2-benzodithiarsole (see (1)), is a useful derivative for GC/MS analysis. Its mass spectrum is simple with molecular ion peaks at m/z 290/292 and the base peak at m/z 229 due to the loss of the 2-chlorovinyl group⁽³⁰⁾.



The reaction product of arsenic trichloride (see Table 1) with 3,4-dimercaptotoluene, 2-chloro-5-methyl-1,3,2-benzodithiarsole, still contains an active chlorine atom, rendering its determination by GC/MS difficult. The derivatization reaction can also be carried out with 2-chlorovinylarsenic oxide (lewisite oxide, CAS 3088-37-7), which is one of the degradation products of lewisite 1. Thus, the highly reactive arsenous compounds can be detected as less reactive derivatives amenable to GC/MS.

6 GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS PROCEDURES FOR ENVIRONMENTAL AND SYNTHETIC MATERIAL SAMPLES

6.1 General

GC/MS is the technique best suited to the identification of CW-related compounds. Nanogram amounts of these compounds are conveniently separated by GC and are then used to produce a full mass spectrum. Complex mixtures can be analyzed within hours. The use of GC for the analysis of CW agents in several matrices has been covered, up until 1990, in an extensive review⁽³¹⁾. Although this review focuses on chromatography, frequent mention of GC/MS underlines the popularity of the method. The state-of-the-art of GC and GC/MS for CW agents and related compounds was later reviewed in 1998⁽³²⁾.

Several types of GC column with different stationary phases can be used for separation. The most popular columns in verification analysis are fused silica columns (typically 0.2–0.3 mm inner dimension (ID), 25–50 m length) coated with the following phases:

- 100 % methyl polysiloxanes (e.g. OV-1TM, SE-30TM, DB-1TM, CPSil5TM, or similar commercial phases);
- 95 % methyl and 5 % phenyl polysiloxane (e.g. OV-101TM, SE-54TM, DB-5TM, CPSil8TM, or similar commercial phases);
- 86 % methyl, 7 % phenyl, 7 % cyanopropyl polysiloxane (e.g. OV-1701TM, CPSil19TM, or similar commercial phases);
- polyethyleneglycol (CarbowaxTM, DB-WAXTM).

Most fused silica columns nowadays show little or no bleeding, resulting in a low MS background. Only the CarbowaxTM phase may produce severe bleeding at high temperatures. Normally, fused silica columns are directly inserted into the ion source of the GC/MS instrument, producing a reduced pressure at the end of the column. The temperature of the transfer line is typically maintained at 250 °C, and helium is the preferred mobile phase. The nonpolar stationary phases, in particular, 95 %

methyl and 5% phenyl polysiloxane, are recommended for analyzing samples containing unknown scheduled chemicals. When unknowns have to be analyzed, temperature programming is the preferred option, with a typical program running from 50 to 250 °C at 10 °C min⁻¹. All common GC injectors, such as on-column or splitless injectors, may be used for GC/MS. A retention gap is sometimes used with on-column injections to prevent contamination of the analytical column with material from the sample matrix. Despite the availability of high efficiency capillary columns, compounds in complex mixtures may not be completely resolved. With a computer program called AMDIS (automated mass spectral deconvolution and identification system), individual component spectra from GC/MS data files can be extracted and then used for comparison with a target database. This program, although applicable to any application, was specially designed and tested for the automatic identification of CW agents and related compounds ⁽³³⁾.

Branching of the *O*-alkyl ester chain of organophosphorus compounds may introduce an asymmetric center, which together with an asymmetric substituted phosphorus atom creates a number of stereoisomers. Diastereoisomers may even be separated on a conventional capillary GC column. This is, for instance, the case with the nerve gas soman, which usually produces two peaks in a gas chromatogram. Although this is characteristic for identifying soman, it also increases the GC/MS detection limit by a factor of two.

Below, sample preparation methods of various types of samples will be briefly discussed. The description is based on sample matrices and is not intended to be comprehensive. Sample preparation techniques originally developed for a specific sample type may, however, also be successfully used for another sample matrix. As a result of international round-robin exercises in the field of verification analysis, a number of recommended operating procedures (ROPs) has been published and tested ⁽³⁴⁾. Further testing of these procedures has taken place since 1995 during proficiency testing under the auspices of the Technical Secretariat of the OPCW. These procedures were developed for the analysis of samples with unknown analytes, in order to allow the identification of as many different chemicals of CWC interest as possible. This approach implies that these procedures can be further optimized in cases

where specific target compounds need to be determined by GC/MS.

Apart from thermodesorption, all other sample preparation techniques are normally directed toward production of a liquid extract that is subsequently injected into a GC/MS system. For the simplest of the samples, neat organic liquids or concentrated solutions, the sample preparation method will consist of diluting the sample with a suitable, clean solvent (most often dichloromethane).

6.2 Air Samples

Air samples are normally collected on polymeric adsorbents such as Tenax[™] or XAD[™]. Tenax[™] tubes are analyzed in the most efficient way by on-line thermodesorption combined with GC/MS. The technique performs very well for thermostable relatively volatile scheduled compounds such as sarin, soman, and mustard gas ⁽³⁵⁾. High boiling nerve agents such as VX may cause problems due to their highly adsorptive nature. Typical desorption conditions imply the heating of the adsorption tube to 200 °C for 10 min in a specially designed thermodesorption injector in a helium flow, and collecting the desorbed volatile compounds in a preconcentrator (a cold trap or the GC column at subambient temperature). By automatic thermal desorption of Tenax[™] tubes followed by GC/MS analysis, 21 compounds of CWC interest (including less volatile agents such as VX) were retrospectively identified ⁽³⁶⁾. Typical detection limits were 50 ng on the tube for most agents in full scan mode. Additionally, optimal storage conditions of the tubes were investigated and the technique of air sampling followed by thermodesorption-GC/MS was validated in field trials with mustard gas.

To overcome problems with VX, a reaction with silver fluoride impregnated on a filter in front of the adsorption tube has been applied. The fluoride converts VX to the more volatile ethyl methylphosphonofluoridate (CAS 673-97-2), which is more amenable to GC ⁽³⁷⁾. In an alternative approach, adsorption tubes can be extracted with a suitable solvent such as ethyl acetate or acetone (chlorinated solvents may dissolve the polymeric adsorption material). This offers the advantage of a repeat analysis. To compensate for the loss of sensitivity, compared with thermodesorption, on-column

injections of large volumes, up to 0.5 mL, could be considered. This requires a GC/MS instrument equipped with a solvent vapor exit system, in order to maintain the mass spectrometer vacuum and to prevent contamination of the ion source.

Nitrogen-containing compounds with basic groups can successfully be trapped on silica. This implies for nitrogen mustards as well as for their precursors. As an example, triethanolamine (CAS 102-71-6) was detected in air at low levels by trapping on activated silicagel (ORBOTM 53 tubes), desorbed and trimethylsilylated prior to GC/MS analysis. Using the SIM mode at m/z 262 ($[M - CH_2-O-TMS]^+$), a detection limit of 1–2 pg was reached for the method ⁽³⁸⁾.

6.3 Synthetic Materials

Scheduled compounds contaminating materials, such as rubber, polymers, paint, clothing, charcoal, and concrete are normally extracted with two solvents of different polarity. In the course of an international interlaboratory comparison test, acetone and dichloromethane have been evaluated for the extraction of nonpolar scheduled compounds, and water has been evaluated for the extraction of polar degradation products ⁽³⁴⁾. Sonication for 5 to 15 min is usually sufficient to obtain good extraction efficiencies. Increasing the surface by cutting or crushing the sample into smaller pieces will enhance the efficiency. The amount of solvent used will depend on the sample, because some samples may absorb relatively large quantities of solvent. As a rule, 1 g of a material needs to be extracted with a few milliliters of a solvent. In general, hydrophobic polymeric materials such as rubber and paint will preserve nerve agents and vesicants to a certain extent. Concrete contains alkaline salts that will hydrolyze nerve agents and vesicants. Therefore, analysis of concrete requires extraction and determination of polar degradation products. This will imply a derivatization step before the GC/MS analysis.

An alternative to solvent extraction of synthetic materials is supercritical fluid extraction (SFE). The SFE recoveries by using carbon dioxide for a series of alkyl alkylphosphonofluoridates and dialkyl alkylphosphonates from alkyd painted plates, compared favorably with conventional sonication results by using dichloromethane ⁽³⁹⁾. A drawback to

this approach is the use of special, rather expensive extraction equipment.

Polymeric material samples contaminated with relatively volatile scheduled compounds such as sarin and mustard gas may also directly be subjected to thermodesorption analysis like that employed for air samples ⁽⁴⁰⁾. In Figure 7(a), part of the resulting total ion current (TIC) chromatogram of a GC/MS analysis is shown for a rubber material contaminated with diesel fuel and the vesicant mustard gas and its impurity mustard disulfide (CAS 1002-41-1). Despite the complexity of the mixture, the vesicants could be positively identified, by extracting pieces of rubber with dichloromethane and subsequent GC/MS analysis of the extract. Polymeric materials, especially rubbers, may contain relatively high (>10 %) quantities of additives (e.g. plasticizers), which may interfere with the GC/MS analysis. Better results were obtained after thermodesorption (see Figure 7b) because the coelution of the large amount of high boiling hydrocarbons is prevented, with concomitant improvement in the GC performance.

6.4 Water Samples

Of the CW agents on the Schedule 1 list, only the nerve agent VX has some stability in water at low pH. Nerve agents of the sarin and tabun families, and especially vesicants such as mustard gas and lewisite 1, hydrolyze relatively quickly. Therefore, water samples must be analyzed not only for the scheduled compounds themselves but also for their polar decomposition products (e.g. methylphosphonic acids, thiodiglycol). Some of the hydrolysis products of well-known CW agents, such as tabun and lewisite 1, are unfortunately not placed on the CWC Schedule list, and up until now their mass spectra or those of their derivatives have not been incorporated in the OPCW Analytical Database. Of the possible degradation products of mustard gas, only thiodiglycol is scheduled, whereas 19 sulfur-containing compounds have been identified by GC/MS upon decomposition of munitions-grade mustard gas in water ⁽⁴¹⁾. Although most decomposition products have not been scheduled, the determination of their presence in water provides valuable information about the original presence of scheduled compounds. Water samples can be collected from the

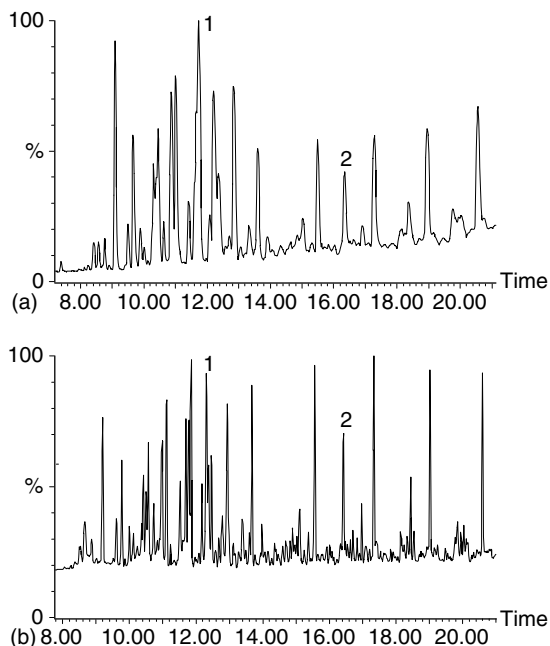


Figure 7. Part of a TIC chromatogram obtained after the GC/MS analysis of rubber contaminated with diesel fuel and sulfur vesicants. (a) Dichloromethane extract and (b) thermodesorption at 120 °C. 1, mustard gas; 2, mustard disulfide. Data recorded at TNO-PML on a VG 70-250S GC/MS instrument (Micromass, UK) ⁽⁴⁰⁾. (Reproduced from Wils, E. R. J., Hulst, A. G., de Jong, A. L., *J. Chromatogr.*, 625, 382–386 (1992) by permission of Elsevier Science)

environment or from waste tanks at chemical facilities. Measurement and adjustment of the pH with either ammonium hydroxide or HCl will be a necessary step during the sample preparation, especially in the case of waste tank samples.

Although LC/MS is a more favorable technique for the analysis of polar degradation products in water samples, GC/MS is still required to provide the additional information for unequivocal identification. GC/MS analysis of water samples is frequently preceded by the following sample preparation approaches:

- liquid–liquid extraction with dichloromethane at three different pH levels (low, neutral, and high) and subsequently either direct analysis of the organic phase or analytical derivatization of the extract;
- isolation of nonpolar compounds using solid phase extraction (SPE), on a cartridge containing a suitable adsorbent (e.g. C₈-, C₁₈-silica or XAD-4TM), or solid phase micro-extraction (SPME);
- isolation of alkylphosphonic acids by an ion-exchange column (e.g. SAXTM cartridge) followed by extraction with methanol, and, subsequently, derivatization of the extract;
- evaporation to dryness followed by derivatization, with subsequent redissolution of the derivatives.

The sample size will be typically 5–10 mL, although applying SPE or ion exchange sometimes allows the use of larger volumes. For instance, the breakthrough volume of nonpolar analytes such as soman or VX on a conventional C₁₈-silica cartridge is over 100 mL.

Among the above-mentioned methods, SPME is relatively new. It combines sample preparation and injection of the sample into one step. Analytes are adsorbed on a polymeric fiber coated with a stationary phase such as polydimethylsiloxane, which is thermally desorbed in the injection port at 250 °C. The successful use of the technique for the GC/MS analysis of the nerve agents in water has been described ⁽⁴²⁾. Levels of less than

60 $\mu\text{g mL}^{-1}$ could be detected under SIM conditions. SPME was also applied prior to the GC/MS analysis of degradation products of CW agents after the in-situ derivatization with MTBSTFA⁽⁴³⁾. This shows that SPME provides a valuable addition to the established extraction methods. SPME fibers have also been successfully used for the collecting of vapors of CW agents from air (e.g. sarin), illustrating that sample preparation techniques prior to GC/MS analysis are applicable to several matrices⁽⁴⁴⁾.

In addition to the above-mentioned more general methods aimed at the GC/MS identification of previous unknown CW agents or their decomposition products, more specific procedures have been developed and optimized for the determination of one specific compound at a low level. For instance, thiodiglycol was isolated from groundwater by sequential SPE with both C_{18} and Amborsorb 572TM columns. The C_{18} column removes extraneous groundwater components, whereas the Amborsorb 572TM column (a synthetic carbonaceous sorbent) adsorbs the polar thiodiglycol. Combined with MTBSTFA derivatization and GC/MS analysis in the SIM mode at m/z 293, corresponding with the $[\text{M} - \text{C}_4\text{H}_9]^+$ peak in the mass spectrum of the derivatized thiodiglycol, a detection limit

between 4 and 16 $\mu\text{g L}^{-1}$ groundwater could be reached⁽⁴⁵⁾.

Decontamination ('decon') solutions result after the destruction of chemical weapons by chemical neutralization with organic solvents or bleach. The resulting aqueous decon solutions need to be analyzed for the original CW agent at a low level before disposal, and may pose a significant challenge to the analyst in comparison with the analysis of CW agents in environmental waters. A well-known decon solution is DS-2 consisting of 2-methoxyethanol, diethylenetriamine, and sodium hydroxide (approx. 1 %). Nerve agents react with 2-methoxyethanol under the formation of esters, which can be readily isolated by liquid-liquid extraction with dichloromethane from aqueous solutions. As a typical example, the EI mass spectrum of the reaction product of VX with 2-methoxyethanol is presented in Figure 8. The spectrum of ethyl 2-methoxyethyl methylphosphonate (MW 182, CAS 170082-62-9) shows no molecular ion peak; the highest peak in the spectrum at m/z 152 corresponds with a loss of formaldehyde. In addition to peaks characteristic for an alkyl methylphosphonate (e.g. m/z 97), strong peaks related to the 2-methoxyethyl moiety (m/z 45 and 58) are present.

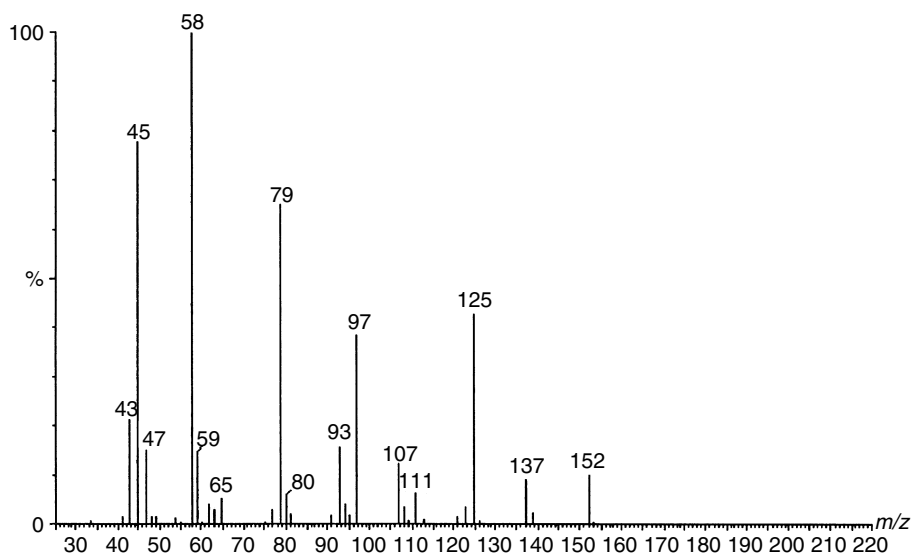


Figure 8. EI mass spectrum of ethyl 2-methoxyethyl methylphosphonate recorded at TNO-PML (Rijswijk, The Netherlands) on a VG Quattro II GC/MS instrument (Micromass, UK)

6.5 Soil and Vegetation Samples

For the purpose of extraction, soil can be considered to be a solid material that may contain relatively large amounts of water (e.g. clay). The sample preparation methods described for solid synthetic materials and water can often be applied prior to the GC/MS analysis. Soil samples are extracted with a nonpolar solvent (e.g. dichloromethane) and a polar solvent (e.g. demineralized water at pH 7). For nitrogen-containing compounds, such as the decomposition products of the nerve agent VX and nitrogen mustards, extraction with water/methanol at pH > 11 is recommended. Alternatively, methanol containing 1 % triethylamine may be used as basic extractant. If the soil contains large quantities of water, nonpolar solvents cannot easily penetrate the pores. In those cases, extraction with a solvent that is more miscible with water (e.g. acetonitrile) is often more efficient. Centrifugation and/or filtration to remove small particles will normally be required before the GC/MS analysis is carried out. It is also recommended that the extract be dried (e.g. with sodium sulfate) and that concentration of the extracts be considered when scheduled compounds are not detected. GC/MS analysis of nonpolar scheduled compounds from soil is typically preceded by a double extraction of 10 g of the soil sample with 10 mL of dichloromethane (shaking or sonication, during 10 min), and filtration of the combined liquid extracts over a microfilter (0.5–1.0 μm). GC/MS analysis of polar scheduled compounds from soil is typically preceded by water extraction, filtration and centrifugation, cation removal of the extract, and derivatization of the residue. Recoveries of the aqueous extraction of the decomposition products of nerve agents may be quite low, in particular, for methylphosphonic acid, and strongly dependent on the soil characteristics. The recoveries of methylphosphonic acid and three alkyl (ethyl, isopropyl, and pinacolyl) methylphosphonates have been determined on 21 different soil types. In addition, the yield of the necessarily TBDMS derivatization prior to the GC/MS analysis was determined ⁽⁴⁶⁾.

Similar to water samples, more specific procedures have been developed and optimized for the determination of one specific compound instead of

the more general methods aimed at the GC/MS identification of previous unknown compounds. These optimized procedures may combine extraction, derivatization, and isolation prior to the GC/MS analysis. For instance, lewisite oxide was determined in soil at a level of 0.1–0.5 $\mu\text{g g}^{-1}$ by extracting soil with an aqueous ascorbic acid solution containing 1,3-propanedithiol as the derivatizing reagent. The resultant formed, 2-(2-chloroethenyl)-1,3,2-dithiar-senane, was extracted from the water phase by a polydimethylsiloxane SPME fiber, which was thermally desorbed in the GC/MS inlet ⁽⁴⁷⁾.

Extraction from vegetation may produce complicated sample extracts, because organic plant material (fats, waxes, resins, etc.) will be dissolved as well. Before GC/MS analysis of these samples, a cleanup step is usually performed to remove compounds originating from the matrix. Injection of resins, typically organic acids, could degrade the GC performance dramatically.

7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS PROCEDURES FOR BIOMEDICAL SAMPLES

During investigations of alleged use of CW agents, samples from biomedical origin may be collected for further analysis in at least two OPCW-designated laboratories. The analysis of biomedical samples, such as blood, urine, tissues, and skin, for the presence of scheduled compounds (or their degradation products) generally poses large problems. These problems are due to a lack of knowledge of the fate of most scheduled compounds in biological matrices, usually low concentrations of the scheduled compounds or their metabolites, and interference of the biological matrix. Analysis of biomedical samples still has to be considered a research, rather than a routine application of GC/MS. In contrast to the situation for environmental samples and synthetic materials, no internationally accepted analysis procedures have been established, neither have the procedures described in literature been validated in international collaborative studies nor has analysis of biomedical samples been the subject of proficiency testing within the context of the CWC.

The development of procedures in this field poses the greatest challenge to the analytical chemist. Although GC/MS(MS) still plays a major role in the analysis of biomedical samples, the role of LC/ES/MS(MS) is increasing, because this latter method allows direct determination of the mostly polar and, sometimes, high MW metabolites.

Determination of the intact CW agents in urine or blood may proceed by the methods commonly applied to water samples. Extraction with an organic solvent and subsequent cleanup with a Florisil column is a well-established procedure. Rather volatile, scheduled compounds can often be successfully recovered and purified from biological materials by means of dynamic headspace stripping and subsequent adsorption on TenaxTM tubes; these tubes are then subjected to GC/MS analysis.

After the use of mustard gas in the Iran–Iraq conflict in the 1980s, the fate of mustard gas in animals has been thoroughly investigated and procedures for the analysis of its metabolic decomposition products have been established⁽⁴⁸⁾. Up to 10 metabolites were identified in the urine of the rats, among which were the compounds thiodiglycol and its sulfoxide. The main metabolites 1,1-sulfonylbis[2-(methylsulfinyl)ethane] [$\text{O}_2\text{S}(\text{CH}_2\text{CH}_2\text{SOCH}_3)_2$] and 1-methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane [$\text{CH}_3\text{SOCH}_2\text{CH}_2\text{SO}_2\text{CH}_2\text{CH}_2\text{SCH}_3$], were due to the reaction of mustard gas with glutathione and subsequent conversion of the glutathione adduct by the enzyme β -lyase. Using GC/MS(MS)(SRM), a detection limit of 0.1 ng mL^{-1} urine could be reached, when both compounds were reduced with titanium trichloride to the single analyte 1,1-sulfonylbis[2-(methylthio)ethane] [$\text{O}_2\text{S}(\text{CH}_2\text{CH}_2\text{SCH}_3)_2$]. The hydrolysis products as well as the β -lyase metabolites could be detected in the urine of human victims of mustard gas attacks⁽⁴⁹⁾.

An alternative approach for the analysis of blood samples from the same group of Iranian mustard gas victims has been described⁽⁵⁰⁾. As mustard gas alkylates amino acids in hemoglobin, adducts will be formed, which remain in the bloodstream for some time. Selective cleavage of the alkylated *N*-terminal valine of the α -chain of hemoglobin was carried out by using the modified Edman reagent pentafluorophenyl isothiocyanate. After derivatization of the adduct-derived pentafluorophenylthiohydantoin with heptafluorobutyric anhydride, the

product is analyzed by GC/MS under negative ion CI conditions.

Procedures based on GC/MS for the determination of the lewisite 1 decomposition product 2-chlorovinylarsonous acid (CVAA, CAS number 85090-33-1) in urine and blood have also been developed. In one procedure, CVAA is converted with BAL; the resulting CVAA/BAL product isolated by SPE on a C_{18} -cartridge and further derivatized with heptafluorobutyryl imidazole⁽⁵¹⁾. A later developed procedure is based on the derivatization of CVAA with 1,3-propanedithiol followed by SPME isolation and GC/MS analysis. Using SIM at the molecular ion peaks, the limit of detection was determined at $7.4 \text{ pg per mL urine}$ ⁽⁵²⁾.

Several procedures were developed for the determination of the nerve agents sarin and VX after their use by terrorists in Japan in the mid-1990s. Intact nerve agents can only be measured shortly after exposure. In blood, they bind primarily and extremely rapid to the enzymes acetyl- and butyrylcholinesterase. In principle, organophosphate-inhibited butyrylcholinesterase in human plasma is the most persistent and abundant source for biomonitoring of an exposure. Treatment of sarin-inhibited esterase with fluoride ions (at pH 4), converts the organophosphorus moiety back into sarin. The regenerated sarin can be isolated by SPE on a C_{18} -cartridge and subsequently be determined by GC/MS. This procedure was successfully applied to serum samples from victims of the terrorist attack in the Tokyo subway (1995), and sarin could be detected in concentrations in the range of $0.2\text{--}4.1 \text{ ng mL}^{-1}$ ⁽⁵³⁾. An alternative approach to the detection of sarin bonded to acetylcholinesterase consists of solubilization of the inhibited enzyme from erythrocyte membranes, digestion with trypsin, and release of the hydrolysis products of sarin by alkaline phosphatase digestion. The two hydrolysis products, isopropyl methylphosphonate and methylphosphonic acid, were derivatized to TMS esters, and subsequently measured by GC/MS (EI and CI). In four victims of the terrorist attack in the Tokyo subway, both sarin hydrolysis products were thus identified⁽⁵⁴⁾. A procedure for the retrospective identification of VX metabolites in serum samples was developed and applied to a victim of VX poisoning. The serum sample was divided into an organic layer in which the nonpolar 2-diisopropylaminoethyl methylsulfide was detected.

In the aqueous layer, ethyl methylphosphonate was found after derivatization with MTBSTFA and 1 % *tert*-butyldimethylsilylchloride. Identification was based on EI, isobutane CI and product ion data of the protonated molecular ion peaks of the two metabolites, m/z 176 and m/z 239, respectively ⁽⁵⁵⁾.

Alkyl methylphosphonic acids are prime targets for analyzing metabolites from nerve agents in serum and/or urine. An extremely sensitive procedure involving a number of steps has been developed allowing the detection of femtogram amounts of these acids in serum/urine samples, as well in environmental (soil and water) samples. The procedure is based on the isolation and enrichment of the methylphosphonic acids on an ion-exchange cartridge, the conversion to their pentafluorobenzyl esters, and CID of the derivatized methylphosphonate anions obtained under negative ion CI conditions ⁽⁵⁶⁾. The procedure has not been applied to samples from victims, as yet. An alternative to this procedure is carrying out the pentafluorobenzylation under liquid–liquid–solid phase transfer conditions using a polymer bound tri-*n*-butylmethylphosphonium bromide as a catalyst ⁽⁵⁷⁾.

The development of procedures for the identification of CW agents in biomedical samples is ongoing and existing procedures are continuously improved. Quantization is also an important factor, and an isotope dilution GC/MS/MS method was developed for the quantitative determination of five organophosphorus acids derived from the nerve agents VX, tabun, sarin, soman, and cyclohexyl sarin in urine samples. The acids were isolated and converted into their methyl esters by diazomethane. Detection limits in the low $\mu\text{g L}^{-1}$ were obtained using CID of the protonated molecular ion peaks obtained with isobutane CI ⁽⁵⁸⁾.

8 QUALITY ASSURANCE

To guarantee the quality and the security of the samples and the information derived therefrom by analysis, it will be necessary to establish a strict QA/QC (quality assurance/quality control) program for the GC/MS analysis of chemicals related to the CWC. This program has to be in accordance with an internationally recognized QA standard (ISO (International Standard Organization)) 17 025

or equivalent. QA requirements equally apply to on-site analysis in the field and to off-site analysis in OPCW-designated laboratories. They encompass the whole chain of events, from taking samples to reporting of the analysis results, with special emphasis on sample preparation and GC/MS analytical methods. Proper coding and sealing of samples and extracts, together with the use of chain-of-custody forms, has to guarantee that a precise history of the samples can be presented. Storage of the samples and extracts in locked refrigerators helps to preserve the samples and to prevent any tampering. Furthermore, frequent checks of the performance of the GC/MS instrumentation and analyses of blank and control samples are required. As the amount of material to be identified decreases, the number of possible errors during the identification process increases. Particularly, when analysis at nanogram or a lower level has to be performed, cross-contamination could be prevented by the application of a strict QA/QC.

For the GC/MS analysis of unknown chemicals, more or less universal procedures have to be used, because of the large variety in matrices and scheduled compounds. This requires the use of ROPs rather than of standard operating procedures (SOPs), because ROPs allow modification of procedures in the process of analysis (validation on the job). However, SOPs have to be followed for instrument calibration and validation.

A key factor in the QA program is the performance control of the instrumentation. A number of test compounds have been recommended for checking the performance of a GC/MS instrument for the analysis of scheduled chemicals ⁽³⁴⁾. These include DMMP, DMMP-*d*9, trimethylphosphate, 2,6-dimethylphenol, 5-chloro-2-methylaniline, tri-*n*-butylphosphate, dibenzothiophene, malathion, and methyl stearate. DMMP is a moderately polar compound and is considered to be a good test compound for checking the GC performance. The deuterated form is recommended because it does not give cross-contamination in the analysis of authentic samples. However, because no scheduled chemicals can be brought on-site during an inspection, the use of DMMP-*d*9 has been replaced by trimethylphosphate for on-site analysis. The correctness of the intensity ratios in the EI mass spectra can be verified by means of the test compounds with the different isotopic peaks: 5-chloro-2-methylaniline

(isotope ratio m/z 141/143 should be $33 \pm 5\%$) and dibenzothiophene (isotope ratio m/z 184/186 should be $5.9 \pm 1\%$). In this way, sensitivity, GC performance, and mass spectrum quality (m/z and relative intensity values) of the described mixtures can be checked.

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ABBREVIATIONS AND ACRONYMS

AMDIS	Automated Mass Spectral Deconvolution and Identification System	DSTL	Defence Science Technology Laboratory
BAL	British-anti-lewisite	EI	Electron Impact
BSTFA	Bis(trimethylsilyl)trifluoroacetamide	ES	Electrospray
BZ	3-quinuclidinyl benzilate	GA	Tabun
CI	Chemical Ionization	GB	Sarin
CID	Collision Induced Dissociation	GC	Gas Chromatography
CN	ω -chloroacetophenone	GD	Soman
CS	<i>O</i> -chlorobenzylidenemalononitrile	GF	Cyclohexyl Sarin
CVAA	2-chlorovinylarsonous Acid	HD	Mustard Gas
CW	Chemical Warfare	HN-1	Ethyl Nitrogen Mustard
CWC	Chemical Weapons Convention	HN-2	Methyl Nitrogen Mustard
DF	Methylphosphonyldifluoride	HN-3	Nitrogen Mustard
DIMP	Diisopropyl methylphosphonate	ID	Inner Dimension
DMMP	Dimethyl methylphosphonate	IR	Infrared
		ISO	International Standard Organization
		L	lewisite
		LC	Liquid Chromatographic
		MIM	Multiple Ion Monitoring
		MRM	Multiple Reaction Monitoring
		MS	Mass Spectrometry
		MS/MS	Tandem Mass Spectrometry
		MTBSTFA	<i>N</i> -methyl- <i>N</i> -(<i>tert</i> -butyldimethylsilyl)trifluoroacetamide
		MW	Molecular Weight
		NMR	Nuclear Magnetic Resonance
		OPCW	Organization for the Prohibition of Chemical Weapons
		PA	Proton Affinity
		PFIB	1,1,3,3,3-pentafluoro-2-(trifluoromethyl)-1-propene
		QA	Quality Assurance
		QC	Quality Control
		QL	<i>O</i> -ethyl <i>O</i> -2-diisopropylaminoethylphosphonite
		RI	Retention Index
		ROP	Recommended Operating Procedures
		SFE	Supercritical Fluid Extraction
		SIM	Single Ion Monitoring
		SPE	Solid Phase Extraction
		SPME	Solid Phase Micro-extraction
		SRM	Single Reaction Monitoring
		TBDMS	<i>Tert</i> -butyldimethylsilyl
		TIC	Total Ion Current
		TMS	Trimethylsilyl
		TNO-PML	TNO Prins Maurits Laboratory
		VERIFIN	Finnish Institute for Verification of the Chemical Weapons Convention

VX *O*-ethyl *S*-2-diisopropylaminoethyl
Methylphosphonothiolate

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CHAPTER 12

Liquid Chromatography/Mass Spectrometry in Analysis of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Over the past decade, liquid chromatography combined with mass spectrometry (LC/MS) has evolved from being primarily a research tool, available in a limited number of analytical laboratories, to a robust and widely available analytical and investigative technique⁽¹⁻³⁾. It is now in widespread use in pharmaceutical, food, environmental, and forensic laboratories and is an indispensable tool in biomedical research. At the time of our previous review of this subject⁽⁴⁾, few laboratories were using LC/MS in the analysis of chemicals relevant to the Chemical Weapons Convention (CWC). This was due to a number of factors, two of which were the cost of instrumentation and a perception that the technique was difficult to implement in an analytical laboratory. In the intervening years, the costs of basic LC/MS have been reduced, and the instrumentation has become robust and easier to operate. LC/MS is now a mature technique that should be considered by all laboratories that are required to analyze chemicals related to the CWC.

2 MS INSTRUMENTATION

2.1 Overview

The instrumentation and interfaces that had been used up to 1998 in CWC-related LC/MS analysis were summarized previously⁽⁴⁾. At that time, sources that operate at atmospheric pressure, using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), were displacing their predecessors that used thermospray ionization or continuous flow fast atom bombardment. Atmospheric pressure ionization (API), either ESI or APCI, is now the method of choice in CWC-related analysis and will be the focus of this current review. A small number of recent applications involving alternative types of ionization are also included. For earlier applications of LC/MS to chemical weapons (CW) analysis, using thermospray and other ionization methods, the reader is referred to our previous review⁽⁴⁾. The other major trend has been the increasing availability and ease of use of less-expensive bench-top quadrupole and

ion-trap instruments⁽⁵⁾. These have allowed LC/MS to become available in a much larger number of laboratories. At the same time, technological developments continue to enhance the capabilities of LC/MS instrumentation. Improvements in the design of sources, mass analyzers, and detection systems have enabled increased sensitivity and mass accuracy to be obtained. Time of flight (TOF) mass spectrometers are gradually making an impact on LC/MS. One area of development that is seldom mentioned is the advance in electronics, which has enabled a large reduction in the number of electronic components and connecting cabling. This has led to reduced electronic noise and a corresponding increase in signal-to-noise. A recently published review details developments in LC/MS instrumentation, both in interfaces and mass analyzers⁽⁶⁾.

2.2 Ionization Methods

2.2.1 APCI and ESI

Most instruments with an API source offer a choice of APCI or ESI. Detailed descriptions of these ionization methods are provided elsewhere⁽¹⁻³⁾. Both rely on the formation of ions at atmospheric pressure in a source region separated from the high vacuum mass analyzer. Ions are transported to the analyzer through one or more differentially pumped skimmers or a heated capillary. Both APCI and ESI require aerosolization of the liquid eluent from the liquid chromatography (LC) column. The major difference lies in the method and phase of ionization.

With APCI, the LC eluent is introduced into the ion source region through a heated nebulizer, assisted by a sheath gas and auxiliary gas flow (usually both nitrogen). Ionization is subsequently induced in the gas phase, usually by a corona discharge, and is dominated by ion-molecule reactions in a similar fashion to normal CI. Protonated solvent molecules, H_3O^+ , $[\text{CH}_3\text{OH} + \text{H}]^+$, or $[\text{CH}_3\text{CN} + \text{H}]^+$, transfer protons to analyte molecules with sufficient proton affinity. The eluent composition can affect the ionization process, for example, more compounds can be ionized when methanol is used as organic modifier compared with acetonitrile, because of the lower proton affinity of methanol. Acetonitrile offers some selectivity if the target analytes are basic with high proton affinity.

APCI sources can accept conventional LC flow rates up to ~ 2 ml/min.

ESI relies more on chemistry and ionization in the liquid phase. A simplistic description is that aerosolization from a capillary tip in a strong electric field produces a mist of highly charged droplets. As the solvent evaporates from these droplets, in a counterflow of nitrogen, analyte molecules are protonated or form other adduct ions. As the droplets become smaller, the charge density increases to the point (the Rayleigh limit) at which Coulombic repulsive forces cause them to disintegrate into many smaller charged droplets. By the same process, these eventually reach the point at which the electric field at the surface is sufficient to desorb ions into the gas phase. A major operational difference between ESI and APCI is that ESI can only accommodate flow rates up to $\sim 10 \mu\text{l}/\text{min}$, unless a nitrogen sheath gas is used to assist aerosolization. This modification, usually referred to as *pneumatically assisted electrospray* or *ionspray*, can accommodate flows up to ~ 1 ml/min. With its low flow rates, ESI is very adaptable to miniaturization, and there is a trend toward using micro or capillary columns that operate at $\mu\text{l}/\text{min}$ or nl/min (nanospray) flow rates. These are particularly aimed at applications in which sample volumes are small, such as proteomics. A number of variations in ESI source design and configurations are available for combining with LC ⁽³⁾.

Both APCI and ESI are 'soft' (i.e. energetically economical) methods of ionization, which with most analytes give predominantly protonated molecules or other adduct ions, for example, with solvent molecules in positive ion mode, and M^- or deprotonated molecules in negative ion mode. In the absence of other analytical data, API spectra usually contain insufficient information for unequivocal identification of unknown analytes. Additional structural information can be obtained by inducing fragmentation through collision induced dissociation (CID). With single-stage mass spectrometers, this can be achieved within the source by applying additional voltages (usually referred to as the *cone voltage*) to accelerate ions within the atmospheric pressure region. The sheath and auxiliary gas and/or solvent molecules act as collision gas. With instruments capable of tandem mass spectrometry (MS/MS), a parent ion is selectively fragmented by collision with an inert gas after the first stage of mass analysis. With in-source CID, all ions in the source may be

fragmented, so chemical noise can be high if components coelute from the LC. With tandem MS, selection of the required ion by the first mass analyzer removes much of the chemical noise, resulting in cleaner product ion spectra and much lower limits of detection in trace analysis. Ion-trap instruments allow a variation on MS/MS in that the parent ions are isolated in the trap in the time dimension before CID, and they allow multiple stages of MS/MS (MS^n).

2.2.2 Choice of APCI or ESI

Because of the need for vaporization before ionization, APCI is generally restricted to molecules with molecular mass < 2000 and can be problematic with thermally labile compounds. ESI, in which ionization occurs in the liquid phase, is applicable to molecules with molecular masses up to $\sim 3 \times 10^5$ Da. The conventional wisdom is that ESI is superior for ionic analytes and large molecules such as proteins, which do not readily vaporize, and APCI is superior for nonionic, more volatile low molecular mass molecules. A detailed and instructive discussion of which to choose has been provided by Thurman *et al.* ⁽⁷⁾ in the context of pesticide analysis. ESI and APCI were evaluated for > 75 pesticides and their degradation products. Neutral and weakly basic pesticides, such as organophosphates, triazines, and arylureas, ionized well using either method of ionization, positive APCI generally being the more sensitive. Cationic pesticides (paraquat and diquat) and those that exist predominantly as anions in solution (e.g. sulfonic acids) gave much better responses using ESI (positive or negative). Overall, ESI provided the broadest applicability to pesticide analysis. These general principles can be applied in CW-related analysis. However, the best mode of ionization appears to be partially dependent on instrumental design and should therefore be determined by experiment.

Another factor to consider in the choice of ionization method is the formation of adducts with sodium or other metal ions, in addition to the protonated molecule. Although these may help confirm the molecular mass, they tend to lower the signal-to-noise ratio of the protonated molecule, resulting in higher detection limits in trace analysis and causing problems in quantitative analysis. Sodium

adducts tend to form with positive ESI but not with APCI. Their formation can be reduced by rigorous removal of metal ions from the system. Adducts with protonated solvent molecules $[M + H + CH_3OH]^+$ or $[M + H + CH_3CN]^+$ are often observed with ESI and APCI, particularly for molecules with low proton affinity. In some cases, they can be reduced by careful adjustment of conditions. Adduct formation is much less of a problem in negative ESI and APCI.

2.2.3 Other Ionization Methods

The eluent jet interface is a modification of the particle beam interface but has not been commercialized⁽⁸⁾. It forms an eluent jet by means of inductive heating of the micro-LC effluent and momentum separation in a jet separator. Coupled to capillary or micro LC, it is more sensitive than particle beam and, like the latter, provides searchable spectra similar to those produced by electron ionization (EI) in gas chromatography/mass spectrometry (GC/MS). It can also operate in CI mode. Atmospheric pressure photo-ionization (APPI) is an alternative to APCI⁽⁹⁾. The corona discharge used in APCI is replaced with 10 eV photons from a vacuum UV lamp in the presence of a dopant (usually toluene). It is reported to be more widely applicable than APCI, extending the useful range of LC/MS to lower polarity molecules, for example, polyaromatic hydrocarbons, and may be more sensitive than APCI for some neutral analytes. Other reported advantages are absence of adduct ion formation and reduced sensitivity to matrix ion suppression in comparison to APCI and ESI. It remains to be seen if APPI provides advantages in the screening for CWC-related compounds in complex matrices.

2.3 Mass Analyzers

The majority of LC/MS systems use either quadrupole or ion-trap mass analyzers. Because of the general paucity of fragment ions under routine operating conditions, instruments with true MS/MS capability, which allow CID of selected ions, have a clear advantage for identification and in providing low limits of detection.

Quadrupole mass spectrometers coupled with LC make up a large part of the market.

They are available in a number of different configurations, ranging from research-grade triple quadrupole LC/MS/MS systems to basic, bench-top single quadrupole mass analyzers. Single quadrupole instruments are limited for unequivocal identification of unknown analytes in that CID is limited to 'in source'. As already stated, this produces additional chemical noise and does not resolve individual components when coelution from the LC column occurs. For analysis of complex matrices, in which resolution of analytes is poor, these instruments can lead to false positives⁽¹⁰⁾. Far more flexible are triple quadrupole systems, in which ions are selectively transmitted by the first quadrupole, CID occurs in the second quadrupole (acting only as a collision cell), and the product ions are separated in the third quadrupole. These systems allow a number of different scanning modes (product ions of selected precursor ions, precursor ions of selected product ions, and neutral loss scans) and can provide very low limits of detection in trace analysis. Unfortunately, triple quadrupole systems are still relatively expensive. Ion-trap systems offer a significantly cheaper and more compact MS/MS capability and they are finding increasing use in analytical laboratories. They allow trapping and subsequent CID of selected ions within the ion trap, although scanning is restricted to product ions. Ion traps are generally more sensitive in full scanning mode than quadrupole systems but may be less sensitive in selected ion mode. Although most of the analytical methods used in the authors' laboratory were developed using a triple quadrupole instrument, most have transferred to an ion-trap LC/MS system without major problems. Expensive high-resolution magnetic sector instruments linked to LC have declined considerably in use other than for research purposes. A recently introduced alternative is a triple quadrupole LC/MS/MS that allows a moderate increase in resolution to 0.1 Da (full width half maximum definition), which can provide very low limits of detection in trace analysis. One variant of this instrument provides accurate mass capability with greater dynamic range than TOF instruments.

An important current development is the use of LC combined with TOF instruments. These have the potential of high-resolution capability, accurate mass determination, and very rapid acquisition rates with detection of all ions transmitted by the flight tube. An LC/TOF instrument is being used

in at least one laboratory for CW analysis⁽¹¹⁾. An LC-quadrupole-TOF tandem mass spectrometer is available in at least two Organization for the Prohibition of Chemical Weapons (OPCW) Designated Laboratories, a major use being the identification of protein adducts as biomarkers of CW agent poisoning (see **Chapter 17**). The instrument is very expensive and at present is more suited to biomedical research than to analysis of low molecular mass compounds.

Other more 'exotic' configurations are becoming available, such as linear (2D) ion traps and combined linear ion-trap/Fourier transform ion cyclotron resonance (FTICR) instruments. Linear traps are less prone to space-charging effects and offer higher sensitivity and linear range. When used as the third element of a triple quadrupole configuration, they provide the full-scan sensitivity and MS/MS capabilities of an ion trap combined with the precursor, product, and neutral loss scan modes of a triple quadrupole. Combination ion-trap/FTICR instruments offer extremely high resolution and mass accuracy and are probably more suited to trace analysis in complex matrices and structural characterization than to general screening.

Most of the applications to CW analysis discussed in the following section have been performed using quadrupole and ion-trap LC/MS systems and should be adaptable for use within most analytical laboratories.

3 LC CHROMATOGRAPHIC CONDITIONS

3.1 Stationary Phase

The majority of LC separations in CW analysis use stationary phases that separate analytes on the basis of hydrophobic interactions under reversed phase conditions, that is, they retain hydrophobic analytes more strongly than polar ones. The most commonly used stationary phases are based on 3–7 (commonly 3 or 5) micron-sized silica particles coated with bonded alkyl phases, or polymeric particles such as styrene-divinyl benzene copolymer. Bonded reversed phase silicas in decreasing order of hydrophobicity are C18 (ODS), octyl (C8), phenyl,

and cyanopropyl. Thus, highly hydrophobic analytes such as VX and BZ will be very strongly retained on C18, leading to long retention times; small highly polar molecules, such as methylphosphonic acid and thiodiglycol sulfoxide, are very poorly retained and have very short retention times. Polymeric column packings are now available in small particle sizes and offer efficiencies approaching those of silica-based packings; generally 3- μ m polymeric packings give efficiency close to that of 5- μ m silica packings. Polymeric packings have the advantage of greater stability at extremes of pH and are more inert than most silica-based materials. Anion exchange stationary phases capable of some hydrophobic interactions and porous graphitic carbon have been used to separate phosphonic acids, the degradation products of nerve agents.

3.2 Mobile Phase

In reversed phase systems, water is the weakest solvent whose strength is modified by addition of a water-miscible organic solvent, usually methanol or acetonitrile. For separation of analytes with a range of hydrophobicities, for example, a homologous series of alkyl alkylphosphonic acids, gradient elution is used with increasing proportions of organic modifier to gradually increase the solvent strength. Low concentrations of volatile buffers, acids, salts or bases, are usually added to modify electronic–polar interactions and to optimize ionization. Mobile phases typically consist of water with methanol or acetonitrile as organic modifier, plus 0.05–0.1 % formic acid or trifluoroacetic acid (TFA). Substitution of ammonium formate for formic acid may be used to promote negative ionization as well as positive ionization.

A recent overview of LC/MS provides the following guidelines for mobile phase selection⁽¹²⁾. For ESI, and to a lesser extent APCI, methanol is preferred to acetonitrile as organic modifier, particularly in positive mode. The volatile buffers ammonium acetate or formate (pH \sim 7) are broadly applicable at concentrations \leq 10 mM for ESI, and $<$ 100 mM for APCI. For positive mode, pH $<$ 7 should be used, pH \sim 5 generally preferred, by adding small percentages of formic or acetic acids, or in combination with ammonium formate or acetate. For negative mode, a pH $>$ 7 should be

used, pH ~ 9 preferred, by addition of NH_4OH or tri- or diethylamine, or in combination with ammonium formate or acetate. If ion pair formation is necessary, heptafluorobutyric acid (HFBA) as an anionic reagent, or tetraethylammonium hydroxide (TEAH) as a cationic reagent, are recommended. It should be noted that in some cases so-called wrong-way-round ionization can occur, that is, the production of negative ions in acidic mobile phases and positive ions in basic mobile phases ⁽⁷⁾.

3.3 Column Diameter and Flow Rates

LC columns with internal diameters (ID) from 0.1 mm (capillary) to 4.6 mm (conventional bore) are in use with ESI or APCI. APCI can accommodate flow rates up to ~ 2 ml/min, and use of conventional bore columns is possible; columns of 2.1 mm (narrow bore) are commonly used, typically at flow rates of 0.2 ml/min. Pneumatically assisted ESI (ionspray) can accommodate flows up to ~ 1 ml/min and can be used with conventional bore columns with split eluent, and narrow bore columns. Pure ESI can only accommodate much lower flow rates, and the options are to use the larger bore columns and split the eluent or capillary or microbore (0.3–1 mm) columns with very low flow rates. Advantages of using narrower bore columns are economy of mobile phase use, generation of less toxic waste, and higher sensitivity. As the internal diameter of the column decreases, the analyte concentration in the column increases, resulting in enhanced sensitivity. A quantitative study of the sensitivity increase with microbore columns, using LC/ESI/MS, was reported by Legido-Quigley *et al.* ⁽¹³⁾ In theory, sensitivity gain is inversely proportional to the square of the internal diameter of the column. Thus, in going from a 2.1-mm column to a 0.3-mm column, the theoretical increase is $2.1^2/0.3^2 = 49$ (which may not be fully achieved) ⁽¹³⁾. Better signal-to-noise is usually obtained with the lower flow rates when using ESI.

Disadvantages of microbore columns are that the increase in sensitivity is partially negated by the smaller injection volume that can be accommodated (although volumes up to $\sim 10 \mu\text{l}$ can sometimes be injected onto 0.3 mm columns), internal dead volumes have to be minimized, and there is increased experimental difficulty. Nano/LC/ESI/MS is more applicable to the analysis of very small

samples, for instance, in proteomic analysis, and requires specialist equipment.

For most applications to CWC-related analysis, conventional and narrow bore columns have been used with APCI and pneumatically assisted ESI, using a split eluent if combined with ESI. The TNO Prins Maurits Laboratory routinely uses micro LC, particularly for biomedical analysis.

4 METHOD DEVELOPMENT

The basics of method development for LC/MS were described in our previous review ⁽⁴⁾. Developments in instrumentation in recent years have influenced the ease and speed of method development and, in some cases, introduced additional opportunities for optimization of MS parameters. It is now possible to optimize most MS and MS/MS parameters automatically, such as temperatures, gas pressures, voltages, and collision energy. Variable angle, and even completely adjustable (x, y, z) sources, allow for fine adjustments to be made when greatest sensitivity is required. Use of so-called segmented scans and data-dependent scanning allow the acquisition of data using different MS and MS/MS scan modes in a single LC run. For example, it is possible to acquire full-scan data in both positive and negative ion modes together with product ion spectra of analytes detected.

In general, method development follows a logical progression, but changes made to one part of the system may have repercussions elsewhere. For example, a change in LC mobile phase composition may affect ionization in addition to LC separation.

When the method is targeted at a single analyte, LC and MS conditions can be optimized using that compound. For generic screening, optimization becomes more difficult, and the analyte used for optimization needs to be chosen carefully. In a screening procedure for CW agent hydrolysis products, using LC/APCI/MS, the protonated molecule from pinacolyl methylphosphonic acid was chosen because it gave the weakest MH^+ ⁽¹⁴⁾. With modern data systems, it is often possible to use different parameters in different time windows throughout an LC run, allowing several analytes to be detected under individually optimized conditions. However, for a screening method to be generally applicable,

it is wise to avoid developing an overcomplicated method.

Instrument manufacturers' manuals are the starting point for instrument setup and advice on method development; they usually give examples of suitable conditions. The initial step (assuming a correctly tuned and calibrated instrument) is to obtain mass spectra of the analyte or analytes of interest. Guidance in the choice of ionization mode is available (Section 2.2.2) ^(7,15) but the ultimate choice should be based on experiment and what gives the best solution to the analytical problem. For optimization, a solution of analyte should be introduced in a suitable mobile phase, either by infusion from a syringe pump or by loop injection (manual or automatic), via a T-piece positioned between the LC pump and the API interface, or directly infused for capillary LC. The LC flow is set with regard to the column size to be used, for example, 0.2 ml/min for a 2.1 mm i.d. column. Changes to ionization conditions are made in order to obtain the best response for structurally significant ions, usually $[M + H]^+$ or $[M - H]^-$ in positive and negative ion modes, respectively. If negative ion spectra are obtainable, then methods using negative ion detection are generally more selective and less prone to adduct formation. Parameters to consider for ESI are spray voltage, gas flows, and temperature within the source, and voltages on components of the ion transfer system between the source and the analyzer, for example, skimmer/sampling cone, heated capillary and lenses or multipoles. Similarly for APCI, corona current and vaporizer temperature are optimized together with gas flows, temperatures, and voltages as above. It should be noted that some of these parameters interact and if, say, heated capillary temperature is changed, the voltage applied to the capillary should be reoptimized.

Once suitable ionization conditions have been established, LC separation can be optimized. As with any LC system, attention needs to be paid to column choice, correct tubing diameters, zero dead volume connections, use of guard columns, and mobile phase filtration and de-gassing. Modern LC pumps can deliver reliable gradients at low flow rates but for capillary LC, precolumn flow splitting or specialized pumps may be necessary. Mobile phase composition and pH should be chosen to

produce good chromatographic peak shapes, sensitivity, and adequate separation while also considering the ionization mode and polarity (see Section 3.2). If the final LC conditions are significantly different from those used for the initial optimization of ionization conditions, then the latter may require reoptimization. For example, the analyte may elute at a different mobile phase composition and the resulting mass spectrum may contain more intense adduct ions, for example, $[M + CH_3CN + H]^+$, than previously. The intensity of these adducts can be reduced and that of $[M + H]^+$ increased by appropriate adjustment of the source conditions. In extreme cases, the LC conditions chosen may dictate a complete change in ionization mode.

Whether directed at screening, or quantitation of trace levels of a target analyte, the aim of method development is to produce a reproducible and robust method that is minimally influenced by small changes in conditions or sample composition. In order to meet these requirements, there may need to be a trade-off between optimum MS conditions and optimum LC conditions. Further, the method giving the best results with pure standards may not be the best when analyzing real samples. It should be noted that matrix effects and coelution can suppress the ionization of target analytes, and seriously affect quantitative analysis using API ⁽⁶⁾. Greater attention may then be required to sample preparation and to LC conditions.

5 OVERVIEW OF APPLICATIONS OF LC/MS IN CW ANALYSIS

5.1 Analytes

Kientz and coworkers ^(16,17) have reviewed the general application of chromatographic methods to CWC-related analysis. In our previous review of LC/MS ⁽⁴⁾, the majority of applications were concerned with environmental-type analysis of polar degradation products of CW agents. This is still the major application because LC/MS avoids the requirement to isolate polar analytes from aqueous matrices and the need for derivatization, both of which can be major sources of error in GC/MS analysis ⁽¹⁸⁾. More recently there have been a number

of applications of LC/MS to the analysis of intact agents in aqueous media, mainly nerve agents, and to situations in which both agent and degradation products may be present, for example, in degraded munitions or in decontamination mixtures. A large number of new applications of LC/MS to the analysis of toxins have been reported, some of which have relevance to the CWC, and to the analysis of biologically active peptides. It is not intended to give a comprehensive review of toxin analysis in this chapter but provide selected examples. Finally, LC/MS is a major technique for the analysis of biological markers of CW agent exposure, in matrices such as blood and urine (see **Chapter 16** and **Chapter 17**). A limitation of LC/MS is that some CW agents are too reactive with aqueous solvents and some column phases. Sulfur mustard and lewisites I and II are in this category, and applications of LC/MS to these agents are restricted to their degradation products and metabolites. Most nerve agents hydrolyze sufficiently slowly for LC/MS analysis to be valid, but care has to be taken in not allowing aqueous solutions to stand for long periods.

5.2 Environmental Analysis

5.2.1 Advantages of LC/MS over GC/MS

CW agents in the environment and in decontamination solutions are subject to hydrolysis and oxidation⁽¹⁹⁾. Both of these processes generally lead to more polar and less reactive molecules; examples are alkyl methylphosphonic acids from nerve agents and thiodiglycol and its sulfoxide from sulfur mustard. Although polar degradation products can be extracted from matrices such as soil with nonaqueous polar solvents, water is often the solvent of choice and is the standard solvent included in the Recommended Operating Procedures for CWC-related analysis⁽²⁰⁾. Historically, analysis of degradation products has been performed by GC/MS, usually after derivatization, but the latter has several disadvantages. Firstly, most derivatization procedures for GC/MS require isolation of the polar analyte from water, often requiring concentration of part aqueous solutions to dryness. The latter is one of the most time-consuming parts of the entire analysis. Concentration to dryness can also be a major source

of error, from losses of analyte by evaporation, inefficient derivatization due to residual traces of water and other extraneous materials, and contamination of laboratory equipment during trace analysis. The requirement for volatility for GC/MS analysis, with or without derivatization, also introduces a bias into the analysis and may exclude certain environmental degradation products. Some examples are provided in the following sections. LC/MS analysis may still require a degree of concentration of analytes, depending on the application and limit of detection required, but it can be applied directly to aqueous solutions and extracts. It is also applicable to a much broader range of compounds than GC/MS, particularly using ESI, and is the only method suitable for the more complex toxins and peptides.

5.2.2 Disadvantages of LC/MS

There are certain reactive analytes for which GC/MS is more suitable than LC/MS. All of the vesicants – mustards and lewisite – fall into this category, as also a number of halogenated precursors of nerve agents that are included in the CWC schedules of chemicals. Where GC/MS has a major advantage over LC/MS is in the unequivocal identification of unknown analytes. LC/API/MS produces mainly molecular or quasi-molecular ions with most analytes, and identification by single-stage MS may lead to false positives. CID may produce only a limited number of product ions, depending on the analyte. Rigid bicyclic structures such as 3-quinuclidinol require high collision energy to produce significant product ions, whereas linear molecules with multiple heteroatoms, such as mustard degradation products, produce abundant product ions at low collision energies. As the appearance of CID spectra is partially dependent on the instrument and conditions, and few libraries are available, it is difficult to use LC/MS CID spectra for unequivocal identification other than by comparison with an authentic sample. For the most polar analytes, which are barely retained under reversed phase conditions (e.g. thiodiglycol sulfoxide, methylphosphonic acid), retention time may not be a reliable indicator. In contrast, the OPCW has a large and expanding database of GC/EI/MS spectra. These provide reliable fingerprints for identification, combined with

characteristic GC retention times. Although LC with particle beam ionization MS can provide spectra similar to those produced by GC/EI/MS, this technique is less adaptable to the more polar analytes and has rarely been used in CW analysis.

Until recently, another clear advantage of GC/MS has been the lower limits of detection that are generally achievable for trace analysis. Examples are thiodiglycol, thiodiglycol sulfoxide, and methyl alkylphosphonic acids. GC capillary columns usually provide sharp narrow peaks, and techniques such as NICI in combination with fluorinated derivatives are inherently extremely sensitive. However, the latest generation of LC/MS/MS instruments, including some ion traps, triple sector quadrupole and TOF systems, are at least an order of magnitude more sensitive than their predecessors and may eventually provide equally low limits of detection. In terms of initial cost, they are still likely to be more expensive than GC/MS instruments.

5.3 Biomedical Sample Analysis

Biomedical sample analysis is discussed in detail in **Chapter 16** and **Chapter 17**. LC/MS/MS is being used increasingly for the analysis of free metabolites in urine and blood, particularly with the emergence of more sensitive instruments. It is usually the method of choice for the analysis of CW agent adducts with macromolecules. LC/MS/MS methods, with limits of detection approaching 1 ng/ml or lower, have been reported for metabolites of sulfur mustard, hydrolysis products of nitrogen mustards, and for isopropyl methylphosphonic acid, the major metabolite of sarin. With a few exceptions (e.g. alkylated N-terminal valine on hemoglobin), LC/MS/MS is the major analytical method used for the trace analysis of adducts with proteins and DNA. Examples are adducts of sulfur mustard with histidine residues in hemoglobin, a cysteine residue in albumin, and the N7-guanine in DNA, and adducts of nerve agents with cholinesterase. LC/MS/MS analysis of protein adducts is usually performed after digestion of proteins to short adducted peptides or individual amino acids. It is also the major research tool used in the initial identification of these adducts.

5.4 Application of LC/MS in OPCW Proficiency Tests

5.4.1 LC/MS for Screening

OPCW proficiency tests currently require the identification of between six and nine analytes (scheduled compounds or their degradation products) in matrices such as water, soil, and organic extracts. Identification must be confirmed by two independent techniques, and both identification and reporting must be in accordance with strict criteria. Although not mandatory, most laboratories rely on comparison with authentic compounds to confirm identification in order to minimize any chance of a false positive. If the authentic compound requires synthesis, then these tests are demanding in terms of time as well as in terms of detection and identification.

In the authors' and several other OPCW-designated laboratories, LC/MS is used as the initial screening procedure for water samples and aqueous extracts of matrices such as soil. This usually provides a tentative identification of polar analytes within half a day, on the basis of molecular mass, any fragment ions present, and retention time. A second analysis, under LC/MS/MS conditions, usually provides a firmer identification on the basis of a limited number of product ions, most of which result from simple neutral losses. With clean matrices, the initial screening may be performed even faster using flow injection or infusion rather than LC⁽¹⁴⁾. An example of the application of flow injection with electrospray ionization/mass spectrometry (ESI/MS) in an OPCW proficiency test is provided by Hooijschuur *et al.*⁽²¹⁾ The identification of the analytes is usually confirmed by GC/MS (in most cases after derivatization) as the second technique.

5.4.2 LC/MS for Identification

The Technical Secretariat of the OPCW has provided rigid criteria for the unambiguous identification of compounds relevant to the CWC⁽²²⁾. These are primarily aimed at the levels used in OPCW Proficiency Tests, in which analytes are typically present at concentrations from 5 to 50 ppm, and full scanning MS is used for identification.

The OPCW requires two independent techniques for identification. In the case of GC/MS, EI, and CI spectra, acquired from separate chromatographic runs, are regarded as independent techniques. A combination of the two is accepted as unequivocal identification – one as a fingerprint, the other to confirm molecular mass. LC/MS provides an alternative to GC/CI/MS for confirmation of the molecular mass and LC/MS/MS provides a partial fingerprint. However, LC/MS/MS spectra, which are normally acquired under identical chromatographic conditions as LC/MS spectra, are not currently considered as a second independent identification. Further development of these criteria may be required with instrumental development. LC retention time is accepted as a second technique if the retention time falls within a window of ± 0.2 min of the retention time for an authentic chemical, with a signal-to-noise ratio of at least 5:1. However, great care has to be exercised if identification is based solely on LC/MS.

The requirements currently stipulated for reporting LC/API/MS data are:

- (a) a total ion chromatogram (TIC) or extracted ion chromatogram (EIC) of a pseudo molecular ion/adduct ion, demonstrating the absence of analyte in a blank sample;
 - (b) a similar TIC or EIC demonstrating detection of the analyte in the spiked sample;
 - (c) an API mass spectrum from the sample showing a pseudo molecular/adduct ion, with the ion indicated;
 - (d) an API mass spectrum of a reference chemical, or of a closely related chemical for the purpose of spectral interpretation.
- (b) an API MS/MS spectrum from the sample with the precursor ion visible and identified;
 - (c) an API MS/MS spectrum of a reference chemical, or of a closely related chemical for the purpose of spectral interpretation.

Additional requirements for MS/MS data when used together with MS data are:

- (a) an API MS/MS spectrum from a sample with the precursor ion visible and indicated;
- (b) an API MS/MS spectrum from a reference chemical, or of a closely related chemical for the purpose of spectral interpretation.

When used alone, requirements for LC/MS/MS data are:

- (a) TICs or EICs of a precursor ion and/or selected fragment ions from the blank and spiked samples;

5.4.3 LC/MS/MS in Trace Analysis

For trace analysis, in which full-scan data cannot be obtained and selected ion monitoring (SIM) or multiple reaction monitoring (MRM) is required, no criteria have been established by the OPCW. Rodriguez and Orescan⁽²³⁾ proposed the following criteria for confirmation of trace levels of pesticides by LC/API/MS under MS conditions that promoted in-source CID:

- (a) LC/MS retention times of the analyte to be within 1 % of that of a standard;
- (b) the molecular ion and two characteristic fragment ions to be present;
- (c) ion abundance ratios of the fragment ions relative to the molecular ion to be within 20 % of the ion ratios obtained for standards.

6 LC/MS ANALYSIS OF NERVE AGENTS, THEIR DEGRADATION PRODUCTS, AND IMPURITIES

6.1 Range of Analytes

The analysis of nerve agents, their degradation products, and related compounds has constituted the greatest number of applications of LC/MS to CWC-related chemicals. This reflects both the importance of nerve agents and the broad applicability of LC/MS to the agents, their degradation products, and common impurities such as dialkyl alkylphosphonates, some of which are also important precursors. The generic structures of nerve agents defined in Schedule 1 of the CWC are shown in Figure 1. The alkyl groups on phosphorus or nitrogen can contain up to three carbon atoms and the alkyl group on oxygen up to 10 carbon atoms. When isomeric variants, degradation products, and related dialkyl alkylphosphonates (Schedule 2B) are considered, these generic structures define more than 1 million

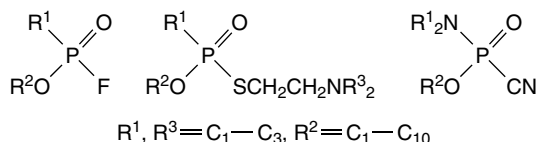


Figure 1. Generic structures of nerve agents defined in Schedule 1 of the CWC

CWC-related organophosphorus compounds. The unequivocal identification of some of these analogues can present a considerable challenge, particularly within the time constraints of an OPCW proficiency test.

6.2 Phosphonic Acids

6.2.1 Degradation Pathways for Nerve Agents

The hydrolytic pathways for nerve agents are summarized in Figure 2⁽¹⁹⁾. Nerve agents of the alkylphosphonofluoridate class (**1**) (e.g. sarin, soman, cyclosarin) and V agent class (**4**) are hydrolyzed initially to alkyl alkylphosphonic acids (**2**) (also referred to as *alkyl alkylphosphonates*) and then more slowly to alkylphosphonic acids (**3**). V agents also hydrolyze by alternative pathways involving C–O and C–S cleavage to give thioacids (**5**) and (**6**). Acids of type (**5**), for example, EA2192 from VX, are of particular importance because they retain high inherent toxicity (though are much less hazardous with regard to skin contact). Tabun (GA) hydrolyzes by two pathways. P–CN cleavage predominates under basic conditions and P–N cleavage predominates under acidic conditions. Further hydrolysis leads to the uninformative ethyl phosphoric acid, which can arise from the hydrolysis of a number of pesticides and other commercially used organophosphorus compounds, and eventually to phosphoric acid.

6.2.2 Characteristics of ESI and APCI Spectra

The phosphonic acids are strong acids ($\text{pK}_A \sim 2$) and under typical LC conditions exist mostly in the deprotonated form. They are therefore well suited to both negative and positive ionization. Kostianen *et al.*⁽²⁴⁾ reported negative-ion ESI (ionspray) spectra for alkyl methylphosphonic acids (alkyl MPAs), *O*-ethyl methylphosphonothioic acid, and

methylphosphonic acid (MPA), in combination with capillary electrophoresis. Borrett *et al.*⁽²⁵⁾ reported the positive and negative ESI spectra of methylphosphonic acid (MPA), and pinacolyl methylphosphonic acid (PinMPA) derived from soman. Positive and negative APCI and ESI spectra for a number of phosphonic acids were reported by Black and Read^(26,27).

Under low cone voltage conditions, positive ESI spectra for alkyl methylphosphonic acids are characterized in most cases by intense protonated molecules MH^+ , plus concentration-dependent protonated dimers and trimers, $[2\text{M} + \text{H}]^+$, $[3\text{M} + \text{H}]^+$. Adduct ions with sodium, water, methanol, or acetonitrile are also observed to varying degrees. A major structurally informative fragment ion is observed at m/z 97, $[\text{MH} - \text{C}_n\text{H}_{2n}]^+$, or $[\text{MH} - \text{C}_6\text{H}_{10}]^+$ with cyclohexyl methylphosphonic acid (cHexMPA), due to loss of alkene via a McLafferty-type rearrangement (Figure 3); the loss indicates the size of the *O*-alkyl group. This ion is also diagnostic of the alkyl group attached to phosphorus, homologous ions at m/z 111 and 125 being observed for alkyl ethyl- and propylphosphonic acids respectively.

APCI spectra show similar MH^+ and fragment ions but generally exhibit weaker adduct ions, particularly with sodium ions. APCI and ESI spectra of isopropyl ethylphosphonic acid are compared in Figure 4, illustrating the lower abundance of adduct ions with APCI. In the case of PinMPA, the protonated molecule is usually relatively weak and is used for optimizing the conditions in the LC/APCI/MS screening procedure reported by Black and Read⁽²⁶⁾. In-source or low-energy collision cell CID of the MH^+ ions for alkyl MPAs produces abundant fragment (or product) ions $[\text{MH} - \text{C}_n\text{H}_{2n}]^+$, plus a number of weaker fragment ions, for example, m/z 79, $[\text{MH} - \text{C}_n\text{H}_{2n} - \text{H}_2\text{O}]^+$.

MPA is characterized by a protonated molecule at m/z 97, a fragment ion at m/z 79 due to loss of

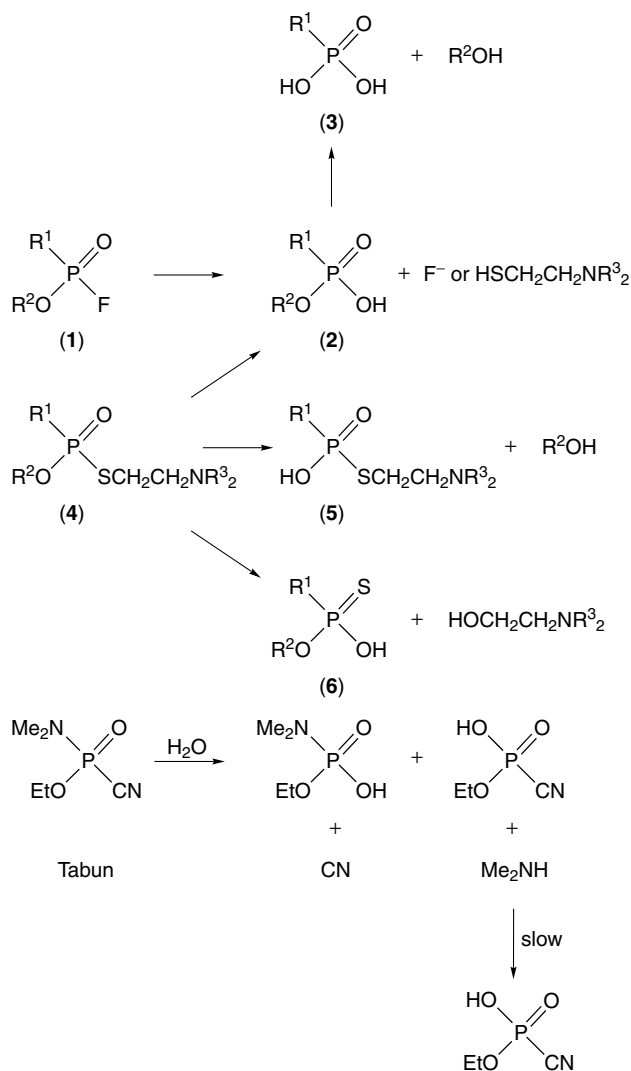


Figure 2. Hydrolytic pathways for nerve agents

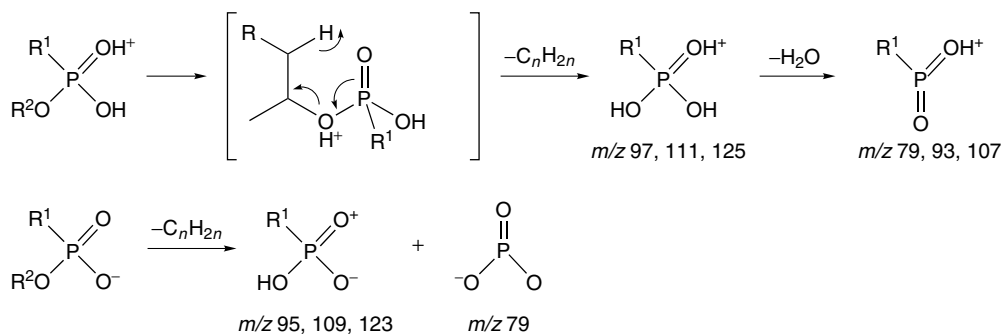


Figure 3. Major fragmentation pathways for alkyl alkylphosphonic acids in positive and negative ion modes

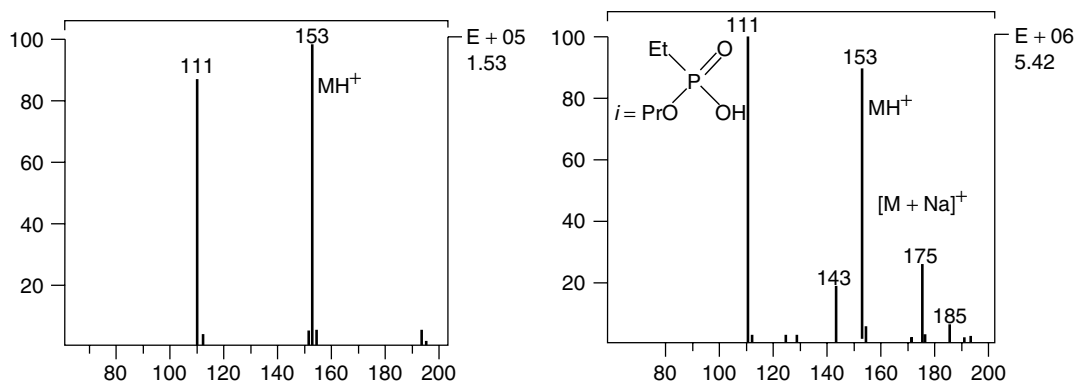


Figure 4. APCI (left) and ESI spectra of isopropyl ethylphosphonic acid, illustrating the lower abundance of adduct ions with APCI; ions at m/z 143 and 185 are adduct ions with MeOH; m/z 175 is $[M + Na]^+$. (Reprinted from Journal of Chromatography A, **794**, R.M. Black and R.W. Read, Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography–mass spectrometry using electrospray and atmospheric pressure chemical ionisation, pp. 233–244 (1998), with permission from Elsevier)

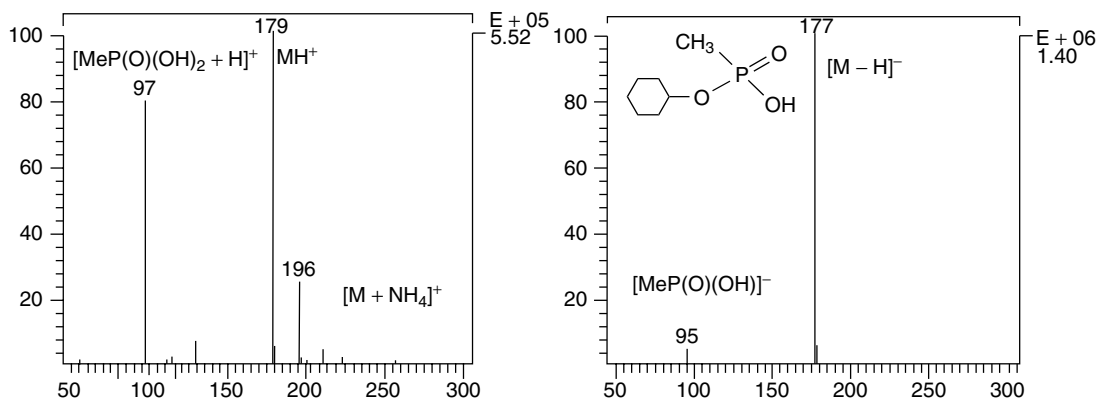


Figure 5. Positive (left) and negative (right) APCI spectra of cyclohexyl MPA

H_2O , and multiple adduct ions using ESI. *O*-Ethyl *N,N*-dimethylphosphoramidic acid, the hydrolysis product of tabun, gave an abundant MH^+ plus polymeric ions⁽²⁸⁾.

Negative ESI spectra are dominated by the deprotonated molecules $[M - H]^-$, and do not exhibit the multiple adduct ions seen in positive mode, though dimeric and trimeric species may be observed. Under higher cone voltages, to promote in-source CID, characteristic fragment ions are observed from loss of alkene $[M - H - C_nH_{2n}]^-$ (m/z 95, 109, 123, etc., depending on the *P*-alkyl substituent) plus an additional ion at m/z 79, probably PO_3^- . The positive and negative APCI spectra of cHexMPA are compared in Figure 5.

6.2.3 Analytical Methods

Borrett *et al.*⁽²⁹⁾, using loop injection, demonstrated the selectivity and much cleaner background obtained when using negative ESI for the analysis of a water sample spiked with MPA. Black and Read^(26,27) reported the first general screening procedures for phosphonic acids using LC/API/MS, both separately and as part of a general screening procedure for CW agent degradation products. An initial method used positive APCI to screen for the degradation products of nerve agents, BZ and mustards⁽²⁶⁾. LC employed a conventional column (250×3.2 mm i.d.) with a mixed C8/C18 stationary phase, and a water-acetonitrile-0.05% TFA gradient

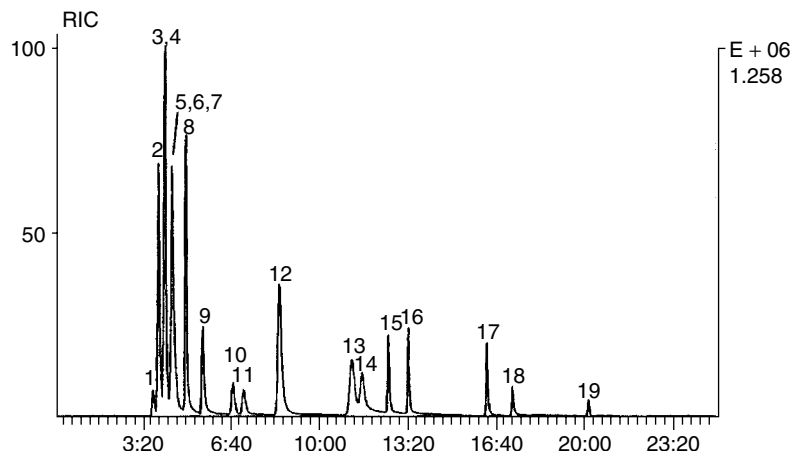


Figure 6. Reconstructed ion current LC/APCI/MS (positive ion) chromatogram of a mixture of 19 analytes (each 10 $\mu\text{g/ml}$ in water). 1. MPA, 2. TDGO, 3. triethanolamine, 4. *N*-methyldiethanolamine, 5. EPA, 6. *N*-ethyl-diethanolamine, 7. thiodiglycol sulfone, 8. 3-quinuclidinol, 9. EMPA, 10. TDG, 11. *n*-PrPA, 12. diisopropylaminoethanol, 13. EEPA, 14. *i*-PrMPA, 15. *tert*-BuPA, 16. *n*-BuPA, 17. cHexMPA, 18. Pin MPA, 19. benzoic acid. (Reprinted from Journal of Chromatography A, **759**, R.M. Black and R.W. Read, Application of liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry, and tandem mass spectrometry, to the analysis and identification of degradation products of chemical warfare agents, pp. 79–92 (1997), with permission from Elsevier)

mobile phase. A reconstructed ion chromatogram (RIC) of a mixture of 19 analytes is shown in Figure 6. For aqueous solutions of standards, SIM of the protonated molecules provided limits of detectability approximately 10 ng/ml for ethyl and isopropyl MPA (EMPA, *i*-PrMPA) and somewhat higher (50 ng/ml) for the more hydrophobic acids, PinMPA and cHexMPA. Poor limits of detection (200–400 ng/ml) were obtained for MPA and ethylphosphonic acid (EPA). Analysis of alkylphosphonic acids is also problematic with GC/MS because of binding to divalent metal ions interfering with derivatization⁽¹⁸⁾.

Greater sensitivity, up to an order of magnitude, was subsequently obtained using positive ESI and substituting 0.1 % formic acid for TFA as the acidic modifier. Zhou and Hamburger⁽³⁰⁾ had previously reported that TFA suppresses and formic acid enhances the formation of $[M + H]^+$ ions for a range of natural products using ESI. Substituting methanol for acetonitrile as the organic modifier led to a reduced intensity of solvent adducts. A disadvantage of these modified conditions was that signal-to-noise ratios were reduced for thiodiglycol and its sulfoxide, and that formic acid as modifier gave poor sensitivity for negative ESI. In order to facilitate

the latter, formic acid was replaced with ammonium formate, which still enabled good sensitivity to be obtained with positive ESI. Chromatographic separation of some analytes was compromised, but, combined with the selectivity of mass spectrometric detection, coeluting analytes could easily be resolved by their mass chromatograms. With increasing experience of the methodology, we observed that APCI was the more robust technique for CW agent degradation products on our triple quadrupole system, probably due to variable adduct ion formation with ESI. The combination of positive and negative APCI, using a C18 column (150 \times 2.1 mm) and water-methanol-0.02 M ammonium formate gradient elution, provided a rapid screening procedure for CW agent degradation products (Figure 7). This is used routinely in the authors' laboratory for OPCW proficiency tests. Limits of detectability using SIM are in the range 10–100 ng/ml for clean aqueous solutions. As observed by Borrett, chromatograms for phosphonic acids are generally cleaner using negative ion than positive ion.

The use of negative ion APCI allows selective detection of phosphonic acids in the presence of neutral and basic analytes. Alkyl alkylphosphonic acids are easily distinguished from isomeric dialkyl

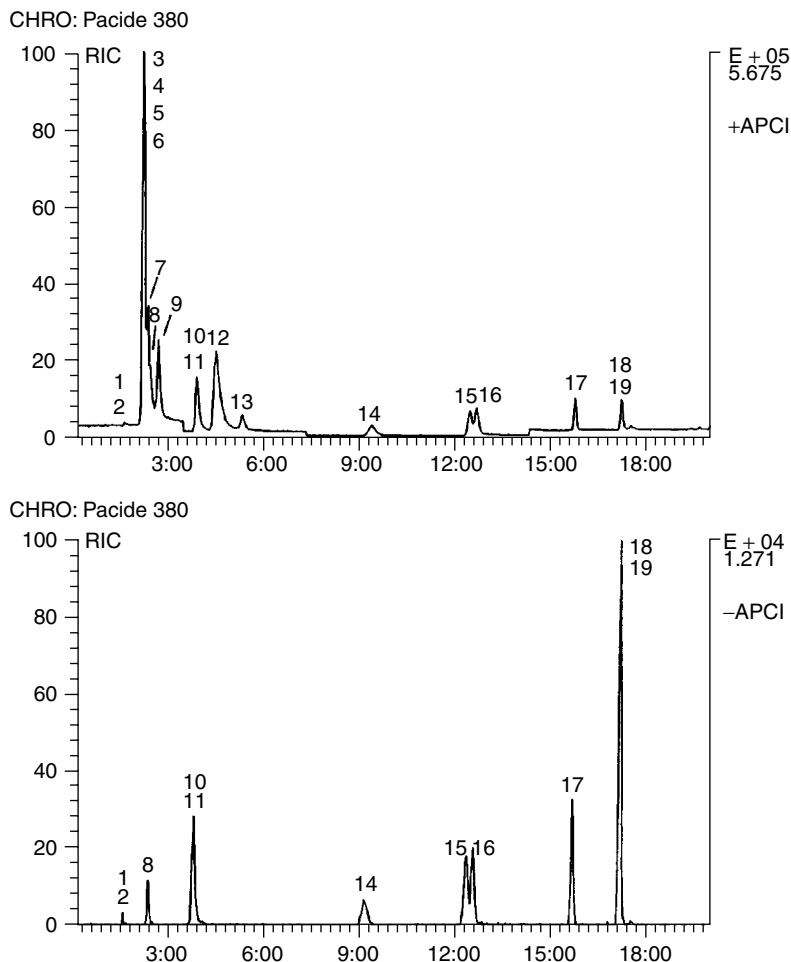


Figure 7. LC/MS selected ion chromatograms, positive APCI (upper) showing the detection of 19 analytes (each 0.1 $\mu\text{g/ml}$ in water), and negative APCI (lower) showing the selective detection of phosphonic acids and benzilic acid in this mixture. 1. MPA, 2. EPA, 3. *N*-methyldiethanolamine, 4. TDGO, 5. 3-quinuclidinol, 6. *N*-ethyldiethanolamine, 7. thiodiglycol sulfone, 8. EMPA, 9. triethanolamine, 10. *i*-PrMPA, 11. EEPA, 12. diisopropylaminoethanol, 13. TDG, 14. *sec*.BuMPA, 15. isobutylMPA, 16. *n*-BuPA, 17. cHexMPA, 18. Pin MPA, 19. benzilic acid. (Reprinted from Journal of Chromatography A, **862**, R.W. Read and R.M. Black, Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography–mass spectrometry with atmospheric pressure chemical ionisation, pp. 169–177 (1999), with permission from Elsevier)

alkylphosphonates (often used in proficiency tests) because the latter give no response in negative ion mode (Figure 8).

The above methods provide good general screening procedures for CW agent degradation products but do not provide optimum chromatographic separation of phosphonic acids. Mercier *et al.* ^(31,32) used a porous graphitic carbon (PGC) column packing for the LC separation of phosphonic acids

in combination with negative ESI (ion spray)/MS and evaporative light scattering detection. PGC is more hydrophobic and more stable than C18 but also undergoes electronic interactions with polar analytes through its delocalized π -electron density. LC used a 150×2.1 -mm column, PGC ($7 \mu\text{m}$) packing, and gradient elution with water-acetonitrile-0.1% TFA. The flow rate was 0.2 ml/min split 1:10 to deliver $\sim 20 \mu\text{l/min}$ to the mass analyzer. Good

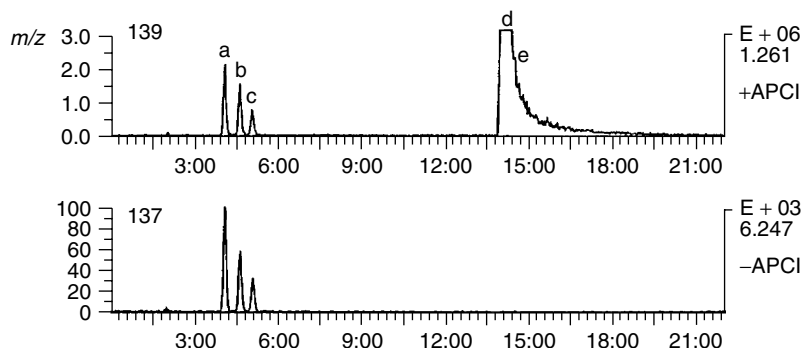


Figure 8. LC/MS mass chromatograms, positive APCI showing the detection of four isomeric alkyl alkylphosphonic acids (1 $\mu\text{g/ml}$) and two dialkylalkyl phosphonates (10 $\mu\text{g/ml}$) (upper), negative APCI showing selective detection of the phosphonic acids. a. *i*-PrMPA and EEPA (unresolved), b. methyl *n*-PrPA, c. *n*-PrMPA, d. ethyl methyl methylphosphonate, and e. dimethyl ethylphosphonate (lower)

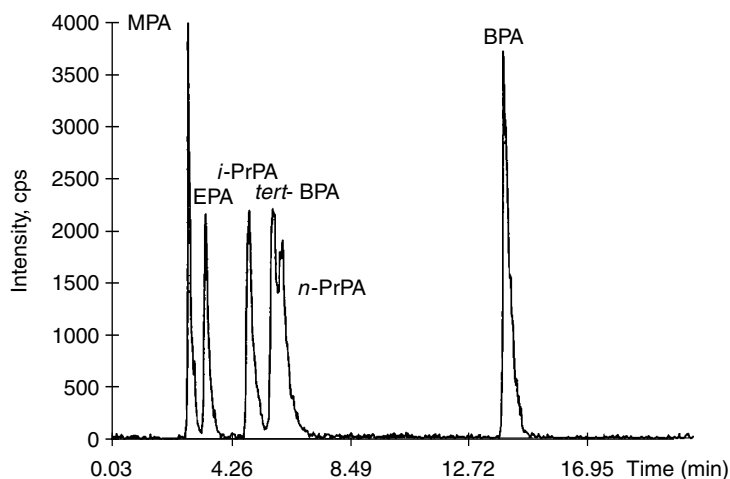


Figure 9. LC/ESI/MS TIC of six alkylphosphonic acids separated on a porous graphitic carbon stationary phase. (Reprinted from Journal of Chromatography A, **849**, Mercier *et al.*, Liquid chromatography analysis of phosphonic acids on porous graphitic carbon stationary phase with evaporative light-scattering and mass spectrometry detection, pp. 197–207 (1999), with permission from Elsevier)

chromatographic separation was achieved for homologous and isomeric alkylphosphonic acids (MPA, EPA, *i*-Pr/*n*-PrPA, *n*-BuPA) and alkyl alkylphosphonic acids. A chromatogram showing the separation of alkylphosphonic acids is shown in Figure 9. The method was used successfully in an OPCW proficiency test (in combination with other techniques) to identify isopropylphosphonic acid and (2-ethylhexyl) MPA.

Polymeric anion exchange columns also provide good separation of phosphonic acids. Hooijschuur

et al.⁽³³⁾ described the use of microcolumn liquid chromatography coupled with FPD, using a PRP-X100 polymeric packing, which possesses both hydrophobic and anion exchange properties.

Siegenthaler⁽³⁴⁾ reported a screening procedure for phosphonic acids, which included ethyl methylphosphonothioic acid and ethyl isopropylphosphonothioic acid, based on negative ion ESI under conditions that promoted in-source CID. LC separation was on a 250 \times 3 mm C18 column, eluted with a water-methanol-20 mM NH_4OAc gradient,

flow rate 0.7 ml/min. In addition to the deprotonated molecules $[M - H]^-$, spectra were characterized by fragment ions $[R^1P(X)(O)OH]^-$ from C–O cleavage and $[P(X)O_2]^-$ ($X=O,S$) from C–O and C–P cleavage. Against a dirty background, for example, PEG 200 in water (commonly used as an obscurant in proficiency tests), the analytes were difficult to distinguish from background peaks when full-scan data was searched using mass chromatograms of the $[M - H]^-$ ions. The acids were much more easily observed by screening the full-scan data for characteristic sets of deprotonated molecules and their fragment ions. Tentative identification could be made on the basis of retention time, molecular mass, and fragment ions resulting from loss of *O*-alkyl and *P*-alkyl groups in comparison with reference data. Limits of detection (LOD) in full-scan extracted ion mode were 20–100 ng/ml, and 5–25 ng/ml in selected ion mode. The method should be even more selective using CID under tandem MS conditions.

Finally, Katagi *et al.* ⁽³⁵⁾ reported the analysis of alkyl alkylphosphonic acids after derivatization to their *p*-bromophenacyl derivatives by LC/MS/MS, using continuous flow frit fast atom bombardment on a hybrid high-resolution magnetic sector-quadrupole instrument. Low ppb (1–20) detection limits were achieved for the acids derived from sarin, soman, and VX using on line SPE/LC/MS/MS, and 0.5–5 ng/ml using single stage LC/MS with SIM of the MH^+ ions. The method is one of the most sensitive of those that use LC/MS. However, it suffers the disadvantage of GC/MS in that it requires concentration of aqueous solutions to dryness before derivatization, and the ionization method, widely regarded as obsolete, is available on few analytical instruments.

6.3 Combined Analysis of Nerve Agents and their Degradation Products

Nerve agents hydrolyze sufficiently slowly to be analyzed by LC/MS under reversed phase conditions. Although analyses for nerve agents alone are usually performed by GC-based methods, there are scenarios, for example, screening of water samples, in which LC/MS offers a rapid alternative. LC/MS is particularly useful for combined analysis of agents and degradation products. A number of applications have recently been reported for the

analysis of degraded nerve agent residues, in which agent, hydrolysis products, by-products, and stabilizers are present.

6.3.1 Sarin, Soman, Cyclosarin (GF), Tabun

D'Agostino *et al.* ⁽³⁶⁾ reported the direct analysis of sarin, soman, cyclosarin, and tabun in aqueous solution in the concentration range 10–100 μ g/ml. LC separation was performed using a 150×0.32 mm ID (5 μ m) packed fused silica column eluted with a gradient of water-acetonitrile-0.1 % TFA. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 200 μ l/min and split prior to the injector to deliver a column flow of 5 μ l/min. Detection was by positive ESI on a hybrid magnetic sector quadrupole instrument (but in single-stage mode). The four nerve agents were well resolved. The ESI mass spectra were characterized by a number of molecular adduct ions, each giving moderate to intense MH^+ , $[MH + CH_3CN]^+$, and $[2M + H]^+$ ions; additional minor adduct ions were observed, for example, $[M + NH_4]^+$ and $[M + H_3O]^+$. Sampling cone voltages of 25–50 V were utilized to promote some in-source CID. The major product ions resulted from alkene loss from MH^+ or the corresponding acetonitrile adduct, for example, m/z 99 $[MH - C_3H_6]^+$ for sarin and m/z 140 $[M + H + CH_3CN - C_6H_{12}]^+$ for soman. Tabun gave weaker fragment ions at m/z 135 $[MH - C_2H_4]^+$ and m/z 176 $[M + H + CH_3CN - C_2H_4]^+$ under these conditions. The intense solvent adducts are probably due to the relatively low proton affinity of the nerve agents; by analogy, they give strong $[M + NH_4]^+$ ions under ammonia CI conditions. Increasing the sampling cone voltage increased the relative abundance of the major product ions due to alkene loss but had relatively little effect on the abundance of acetonitrile adducts. Similar adduct ions with solvent were observed using a quadrupole mass selective detector, and when methanol was used as organic modifier. The sensitivity of the method was of the order of 5 ng for full-scan spectra, acquired during the analysis of a 10- μ g/ml standard.

Using similar LC conditions but employing a sensitive LC/TOF/MS system, the same authors analyzed sarin, soman, and their hydrolysis products in soil ⁽¹¹⁾. Three representative soil types were

spiked with the nerve agents and their hydrolysis products *i*-PrMPA and PinMPA at 10 or 50 $\mu\text{g/g}$. The samples were extracted with water using ultrasonic vibration, centrifuged and analyzed directly. Recoveries of sarin and soman were comparable to those achieved with dichloromethane extraction for GC/MS analysis. Recoveries of the hydrolysis products were superior to an extractive silylation procedure using dichloromethane and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA); some hydrolysis of the agents in the sample on standing or during handling was noted. The only anomaly was peak splitting of the *i*-PrMPA peak in the presence of coextracted soil sample components. The same LC/TOF/MS system was applied to the analysis of snow that was accidentally contaminated with sarin during the destruction of a chemical munition⁽³⁷⁾. Sarin, its hydrolysis products, a number of related compounds (e.g. isopropyl methyl methylphosphonate, diisopropyl methylphosphonate), and amine stabilizers (e.g. tributylamine) were also identified. A resolution of 5000 aided the assignment of elemental composition of the observed ions. Similar studies were reported for munition grade and crude synthetic samples of tabun in which a number of degradation and by-products were identified, including some pyro compounds^(38,39). An ESI/MS database of CW agent-related analytes is now available from the Canadian defense research group⁽³⁹⁾.

6.3.2 V Agents

The positive ion ESI spectrum of VX, which has a high proton affinity by virtue of its diisopropylaminoethyl substituent, gave the protonated molecule as the only significant ion⁽⁴⁰⁾, in contrast to the multiple adducts seen with G agents. In fact, LC/API/MS is a useful alternative to GC/CI/MS as

a second technique for the unequivocal identification of V agents in proficiency tests. Bell *et al.*⁽⁴¹⁾ studied the fragmentation pathways for VX, and the isomeric Russian V agent [*S*-diethylaminoethyl *O*-isobutyl methylphosphonothiolate], by ESI-ion trap MS using sequential CID. CID of the protonated molecule of VX gave two fragment ions, m/z 128 and m/z 167, with an intensity ratio of 20:1. These were assigned to aziridinium ion $[\text{CH}_2\text{CH}_2\text{N}(i\text{-Pr})_2]^+$ and an ion derived from loss of $(i\text{-Pr})_2\text{NH}$. With the Russian V agent, CID of the protonated molecule gave fragment ions at m/z 212 and m/z 195, intensity ratio 25:1, due to loss of alkene C_4H_8 and Et_2NH , respectively. Fragmentation pathways were also investigated for minor impurities $(\text{SCH}_2\text{CH}_2\text{NR}_2)_2$ and $\text{Me}(\text{R}^2\text{O})\text{POP}(\text{OR}^2)\text{Me}$.

The advantages of LC/MS analysis over GC/MS for the characterization of degraded CW agents were demonstrated in the analysis of a degraded sample of VX⁽⁴⁰⁾. Using the system and conditions described above for the analysis of sarin, soman, and cyclosarin, the molecular masses of 38 sample components were established, although not all were resolved by LC. Two thirds of these were tentatively identified by interpretation of the ESI/MS data. Relatively high cone voltages (50 and 100 V) were used to promote in-source CID to produce structurally informative fragment ions. LC/MS identified the agent, impurities, degradation products and stabilizers in a single chromatographic analysis. A number of compounds were tentatively identified that had not been detected in a previous GC/MS analysis of the sample. The range of structures is illustrated in Figure 10. Disadvantages compared to a GC/MS analysis were that less structural information was obtained and the chromatographic resolution of LC was inferior to GC.

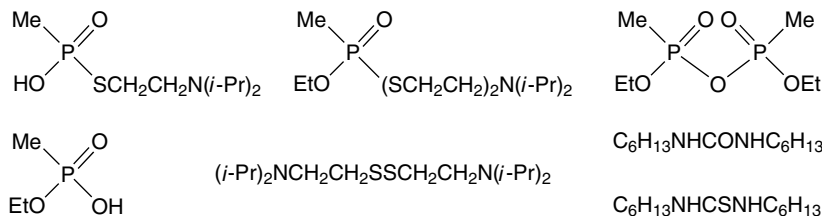


Figure 10. Some components of a degraded VX sample identified by LC/MS

The ability of LC/MS to simultaneously monitor agents and degradation products makes it well suited to following decontamination reactions, together with NMR. In the case of VX, one of the possible hydrolysis products, EA2192, retains much of the systemic toxicity of VX. This degradation product can be monitored by GC after derivatization but it is much simpler to use LC/MS and ^{31}P NMR. Creasy *et al.* ^(42,43) reported the use of LC/APCI/MS/MS and LC/ESI/MS for monitoring the decontamination of VX in caustic monoethanolamine solution. The decontamination mixture was neutralized with acetic acid and diluted before analysis. Using APCI/MS/MS, a 200×2.1 mm C18 column was used, eluted with a 0.05-M aqueous ammonium acetate-acetonitrile gradient. The protonated molecules of both analytes give m/z 128, $[\text{CH}_2\text{CH}_2\text{N}(i\text{-Pr})_2]^+$, as the major CID product ion and these transitions were used for MRM using a triple quadrupole instrument; limits of detection down to 50–100 ng/ml were achieved ⁽⁴²⁾. For LC/ESI/MS using single-stage quadrupole MS, separation was on a 150×2 mm ID reversed phase column eluted with a gradient of water-acetonitrile-0.5–1 % acetic acid, flow rate 0.25 ml/min split 1:5 before entering the mass selective detector. The limit of detection for EA2192 in the diluted reaction solution was 8 ng/ml, with a similar sensitivity for VX ⁽⁴³⁾.

Schedule 1 of the CWC also includes quaternary salts of V agents, in which the side chain nitrogen is quaternized. Although these have generally been neglected as analytes, LC/MS and NMR are likely to be the only analytical techniques suited to the detection and identification of these compounds.

6.4 Dialkyl Alkylphosphonates

Dialkyl alkylphosphonates are of interest as analytes for a number of reasons. They may be present as impurities in nerve agents, and as such may be useful indicators of use or production as they are much more stable in the environment; an example is diisopropyl methylphosphonate found in sarin. Dialkyl methylphosphonates may be formed in decontamination reactions of nerve agents in basic formulations containing alcohols or cellulosives. They are also important precursors to nerve agents, for example, dialkyl methylphosphonates are converted to the key

intermediate precursor methylphosphonic dichloride on treatment with chlorinating agents. Dialkyl alkylphosphonates (*P*-alkyl up to C3, *O*-alkyl up to C10) are captured by Schedule 2B of the CWC and a number of different analogues have appeared in OPCW proficiency tests.

6.4.1 Mass Spectra

Harden *et al.* ⁽⁴⁴⁾ studied the fragmentation pathways of dialkyl alkylphosphonates using ESI under CID conditions. The major pathways are similar to those of alkyl alkylphosphonic acids. When the *O*-alkyl groups are ethyl or larger, CID is dominated by McLafferty type rearrangements with O–C cleavage and neutral losses of alkene. Thus, diisopropyl methylphosphonate is characterized by successive losses of 42 amu (C_3H_6) from the *O*-alkyl substituents to give product ions of m/z 139 and 97. A further ion is observed at m/z 79 as with MPAs. These ions provide a tentative identification, at least in terms of the number of carbon atoms for the *O*-alkyl and *P*-alkyl substituents. In the case of dimethyl methylphosphonate (DMMP), P–O cleavage occurs with neutral loss of methanol ⁽⁴⁵⁾. An interesting difference between an ion-trap and triple quadrupole instrument was observed in the CID spectra of the protonated molecule of DMMP. In the ion trap, a strong ion at m/z 111 was observed derived from addition of background water to the product ion m/z 93 $[\text{MH} - \text{MeOH}]^+$. In the triple quadrupole instrument, in which the residence time in the collision cell is shorter, only a weak ion was observed at m/z 111. Other more complex fragmentations were observed and investigated using deuterium labelling.

6.4.2 Analytical Methods

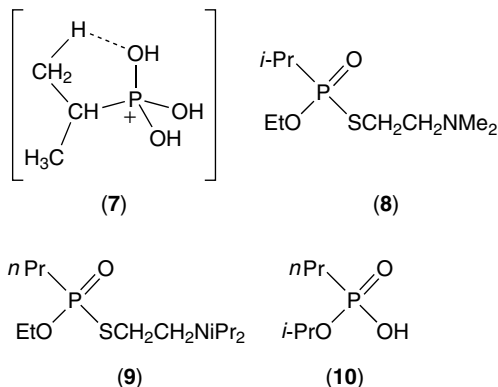
Specific analytical methods have not been developed for dialkyl alkylphosphonates. They are usually sufficiently hydrophilic to be partially extracted by water from matrices such as soil and they are detected by the screening methods described above under positive ESI and APCI conditions. They are easily differentiated from isomeric alkyl alkylphosphonic acids by their lack of response under negative ionization conditions (Section 6.2.3, Figure 8) ⁽¹⁴⁾.

6.5 Distinguishing Isomers

In the generic structures of nerve agents shown in Figure 1, the alkyl groups on phosphorus or nitrogen can contain up to three carbon atoms and the alkyl group on oxygen up to 10 carbon atoms. In OPCW proficiency tests, laboratories are required to specifically identify the propyl isomer on phosphorus (*n*- or *iso*) and nitrogen. Isomeric substituents on oxygen do not have to be identified other than by their elemental composition, but if comparison is to be made with a synthetic standard, then identification of the *O*-alkyl substituent is clearly beneficial. The LC/MS methods as described above do not predictively distinguish compounds with isomeric *O*-alkyl or *P*-alkyl substituents (other than by retention time if standards are available). In proficiency tests, this can result in a laboratory having to spend considerable time in synthesizing a number of alternative isomers.

Van Baar *et al.*⁽⁴⁶⁾ reported that isomeric propyl substituents on propylphosphonic acids could be distinguished using LC/ESI/MS/MS. The ESI/MS/MS spectra of the MH^+ ions of *n*-propyl and isopropylphosphonic acid show two major differences. The *n*-propyl isomer shows an abundant product ion from loss of water, whereas this ion is weak with the *P*-isopropyl isomer. The isopropyl isomer shows a strong product ion due to loss of propene at m/z 83 $[MH - C_3H_6]^+$; deuterium labelling experiments showed that this involves a 1,4 hydrogen shift from one of the isopropyl methyl groups, possibly through a cyclic intermediate (7). This ion was not observed with the *n*-propyl isomer. The isopropyl isomer also showed an intense peak at m/z 65 (further loss of H_2O) that is weak in the *n*-propyl isomer. The method was used to identify two V agent analogues (8, 9) with *P*-propyl substituents and isopropyl *n*-propylphosphonic acid (10) in OPCW proficiency tests. The V agents were partially hydrolyzed to the propylphosphonic acids inside the ESI source, and isopropyl *n*-propylphosphonic acid partially hydrolyzed on standing.

The authors' laboratory has extended this methodology to dialkyl propylphosphonates⁽⁴⁷⁾. Fifteen pairs of isomeric dialkyl propylphosphonates were distinguished without hydrolysis. Differentiation



was based on CID spectra of the ions m/z 125 $[MH - C_nH_{2n} - C_mH_{2m}]^+$, derived from successive losses of *O*-alkyl substituents, or m/z 139 $[MH - C_nH_{2n}]^+$ ions in the case of alkyl methyl propylphosphonates. They were clearly differentiated by loss of propene (also ethene, propanol, and water) from the isopropyl isomers. In no case did the *n*-propyl isomer show a fragment ion at m/z 97 $[M - C_nH_{2n} - C_3H_6]^+$ $([PrP(O)(OH)_2 + H]^+)$, and there were significant differences in the intensities of product ions at m/z 79 and m/z 65. The method successfully predicted the structure of isomers submitted 'blind' for analysis. CID spectra for methyl ethyl *n*- and *iso*-propylphosphonate are shown in Figure 11. Isomeric *O*-propyl substituents could also be differentiated.

6.6 Other Nerve Agent Degradation Products

A number of other nerve agent degradation products can be conveniently detected using LC/MS. V agent hydrolysis products or precursors such as $HOCH_2CH_2N(i-Pr)_2$ and $HSCH_2CH_2N(i-Pr)_2$ are detected in LC/MS screening procedures. In a recent proficiency test, the sulfonic acid $HO_3SCH_2CH_2N(i-Pr)_2$, a product of the oxidative decontamination of VX, was detected in a water sample by LC/MS, although GC/MS after derivatization was used by most laboratories for identification. Dijkstra *et al.*⁽⁴⁸⁾ demonstrated the detection of 2-diethylaminoethanol (and some organophosphorus pesticides) in EI and CI mode using an eluent jet interface coupled to micro-LC.

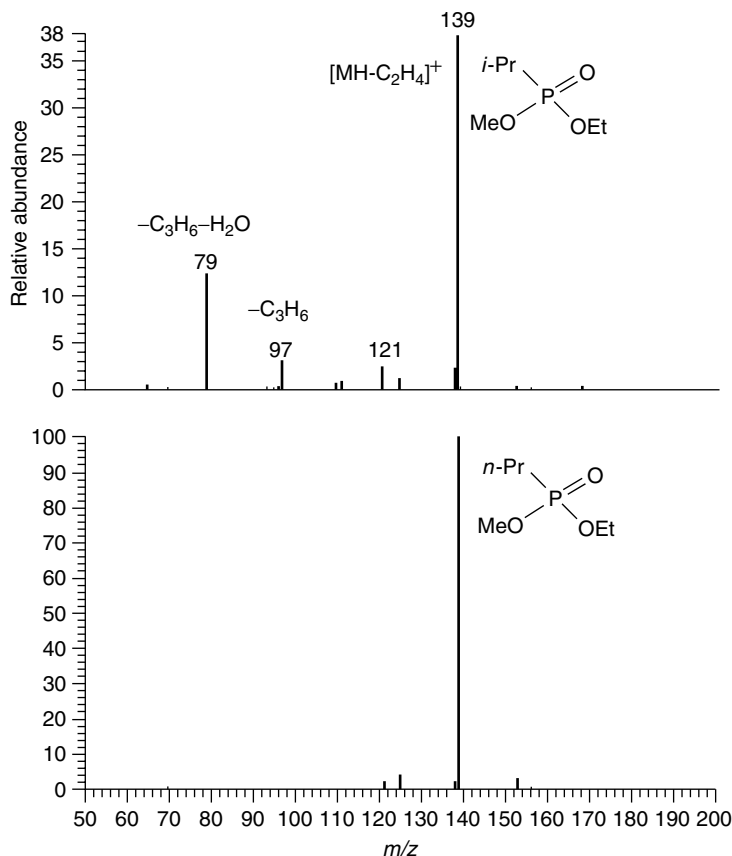


Figure 11. CID spectra (under similar conditions) of m/z 139 differentiating ethyl methyl isopropylphosphonate (upper) from ethyl methyl *n*-propylphosphonate (lower)

6.7 Biomedical Sample Analysis

6.7.1 *i*-PrMPA

Although LC/MS has generally been less sensitive than GC/MS for alkyl MPAs, improved LC/MS/MS instrumentation is enabling lower LODs to be achieved. Noort *et al.* ⁽⁴⁹⁾ reported a sensitive micro-LC/MS/MS (triple quadrupole) method for the detection of *i*-PrMPA in serum. $[^2H]_3$ -*i*-PrMPA was added as internal standard, the solution acidified with dilute sulfuric acid, and *i*-PrMPA extracted with isobutanol/toluene 1:1. A microbore PRP-X100 column was used for LC, isocratically eluted with water-acetonitrile (1:1) plus 0.5 % formic acid. The LOD was 1–4 ng/ml using either positive or negative ion ESI/MS/MS. Fragmentations monitored

were $[M-H]^- \rightarrow [M-H-C_3H_6]^-$ (m/z 137 \rightarrow 95) in negative mode and $MH^+ \rightarrow [MH-C_3H_6]^+$ (m/z 139 \rightarrow 97) in positive mode (plus the corresponding fragmentations from the internal standard). The method was applied successfully to samples from human casualties of the Tokyo subway attack (*see Chapter 16*). A method applicable to serum, using continuous flow frit fast atom bombardment MS ⁽³⁵⁾, is described in Section 6.2.3.

6.7.2 VX

In the authors' laboratory, VX can be analyzed directly in aqueous buffers from microdialyses, for example, of skin perfusates, without preconcentration, down to 0.01 ng/ml using SRM on a

new-generation triple quadrupole instrument. This provides a very sensitive and convenient method in research into improved medical countermeasures.

6.7.3 Protein Adducts

Fidder and coworkers⁽⁵⁰⁾ developed a versatile procedure that identifies phosphorylated butyrylcholinesterase. Adducted butyrylcholinesterase is isolated from plasma by affinity chromatography (procainamide column), digested with pepsin, and a nonapeptide containing the phosphorylated active-site serine residue detected using LC/ESI/MS/MS (quadrupole-TOF hybrid instrument). A C18 150 × 0.3-mm LC column was used, eluted with a gradient of water-acetonitrile-0.2% formic acid. The method was applied successfully to casualties of sarin poisoning from the Tokyo subway attack (see Chapter 17).

Sarin and soman have been shown to bind to a tyrosine residue present in a blood protein⁽⁵¹⁾. The precise site of this residue has not yet been confirmed but it is associated with the albumin fraction. An LC/ESI/MS/MS (triple quadrupole) method was developed for its detection after digestion of the albumin fraction with Pronase and clean up on a C18 SPE (solid-phase extraction) cartridge. A

C18 column (250 × 2 mm) was used for separation using gradient elution with water-acetonitrile-0.05% formic acid.

7 SULFUR MUSTARD

7.1 Intact Agent

Sulfur mustard is generally analyzed by gas chromatographic techniques. When in solution, it reacts rapidly with water (half-life ~5 min in distilled water at 25 °C) and is therefore not suited to reversed phase LC. D'Agostino⁽⁵²⁾ reported that it does not ionize under ESI conditions.

7.2 Sulfur Mustard Hydrolysis Products

7.2.1 Degradation Pathways

In dilute solution, the primary hydrolysis product of sulfur mustard is thiodiglycol (TDG) (Figure 12). In the environment, TDG may be oxidized to its thiodiglycol sulfoxide (TDGO), and more rarely to the sulfone, though the latter may be an important

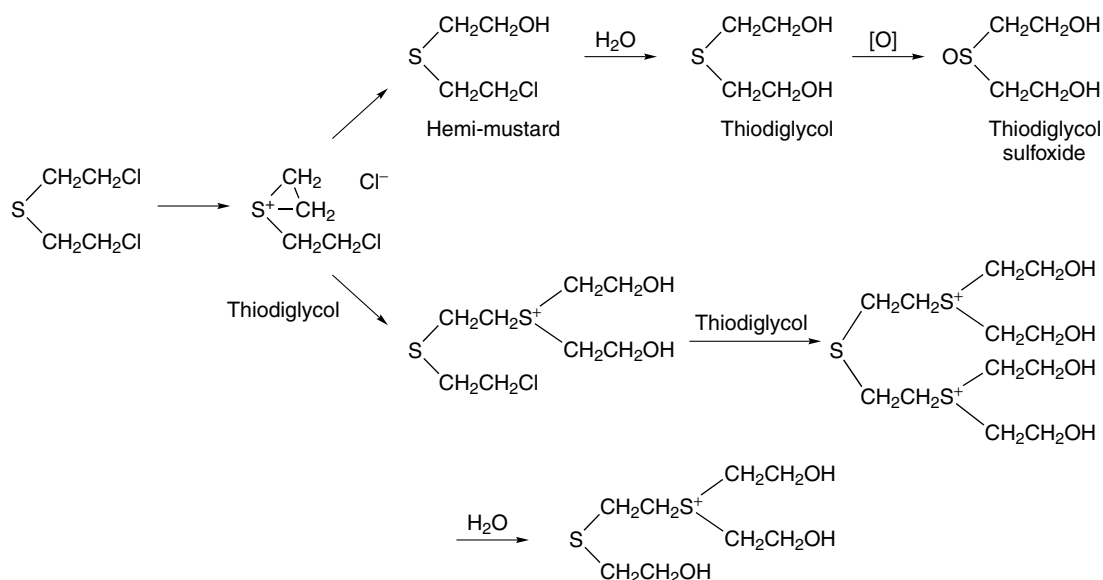


Figure 12. Hydrolytic pathways of sulfur mustard

product of oxidative decontamination. Microbial activity may convert thiodiglycol to thiodiglycolic acid⁽¹⁹⁾. Oligomers of TDG may be produced via sulfonium ion pathways, their formation being favored at higher concentrations of mustard. Part of this process is also illustrated in Figure 12.

7.2.2 Thiodiglycol, Thiodiglycol Sulfoxide and Homologues

General LC/MS screening procedures for CW agent hydrolysis products that include TDG and TDGO are used by a number of laboratories. The ESI and APCI mass spectra of TDG are particularly sensitive to cone voltages and may exhibit an abundant to weak MH^+ , with a strong fragment ion at m/z 105 resulting from loss of water. Strong Na^+ adducts may be observed during ESI analysis⁽²⁹⁾. TDGO gives an intense MH^+ at m/z 139, with a relatively weak ion due to loss of H_2O . Because of the presence of three heteroatoms in a linear chain, CID of the protonated molecules of TDG and TDGO give a number of predictable product ions, resulting from cleavage of C–S and C–O bonds, that provide good fingerprints for identification. Using LC/APCI/MS, limits of detectability in clean water were ≤ 10 ng/ml for both analytes using SIM, TDGO giving the superior signal-to-noise ratio^(14,26). TDG and TDGO

have been readily detected in OPCW proficiency tests using this procedure. LC/MS is particularly useful for TDGO as this analyte may be missed if *tert*-butyldimethylsilyl derivatization is used for GC/MS⁽¹⁸⁾. The APCI screening method^(14,26) has been expanded to include the hydrolysis products of the sulfur mustard homologues (11) and (12) (*O*-mustard, T), which are also included in Schedule 1 of the CWC, and their sulfoxide and sulfone oxidation products⁽⁵³⁾. The APCI spectra of these compounds, with ammonium formate as LC modifier, differ according to the oxidation state of sulfur. Homologues of thiodiglycol gave moderately abundant MH^+ and $[M + NH_4]^+$ ions, accompanied by an abundant ion from loss of water $[MH - H_2O]^+$. Homologues of TDGO gave spectra dominated by MH^+ . The homologues of thiodiglycol sulfone, which presumably have a lower proton affinity, gave $[M + NH_4]^+$ adduct ions as the base peaks. Weak to moderately intense fragment ions were observed, resulting from simple cleavage of C–S and C–O bonds; these could be enhanced by CID. LC/MS has clear advantages over GC/MS analysis for sulfoxide oxidation products, which even when derivatized are prone to elimination reactions on hot surfaces. Furthermore, the intense high mass ions observed with APCI and ESI are readily identified as containing sulfur by their ^{34}S isotope $[MH + 2]^+$ ions. Figure 13 shows a TIC demonstrating the

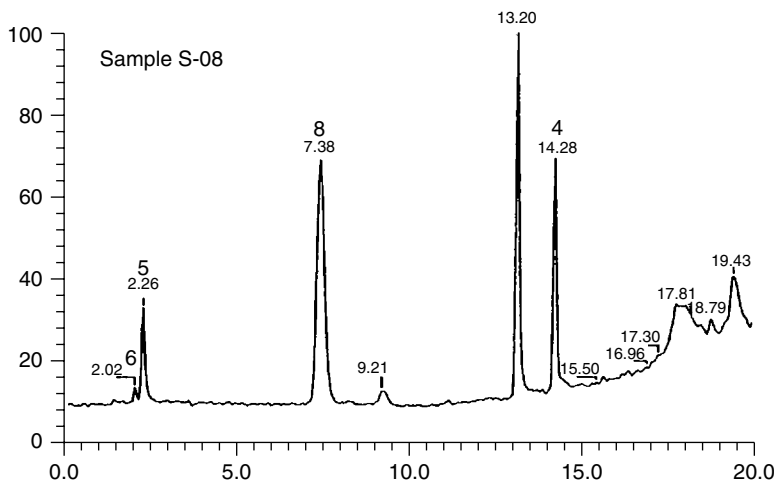


Figure 13. TIC chromatogram of a water extract of a proficiency test soil sample showing the detection of 6. TDGO (from degradation of TDG), 5. ethyl MPA, 8. 1,5-bis(2-hydroxyethylsulfanyl)pentane (13), 4. dimethyl ethylphosphonate (additional intense peak is trimethyl phosphate)

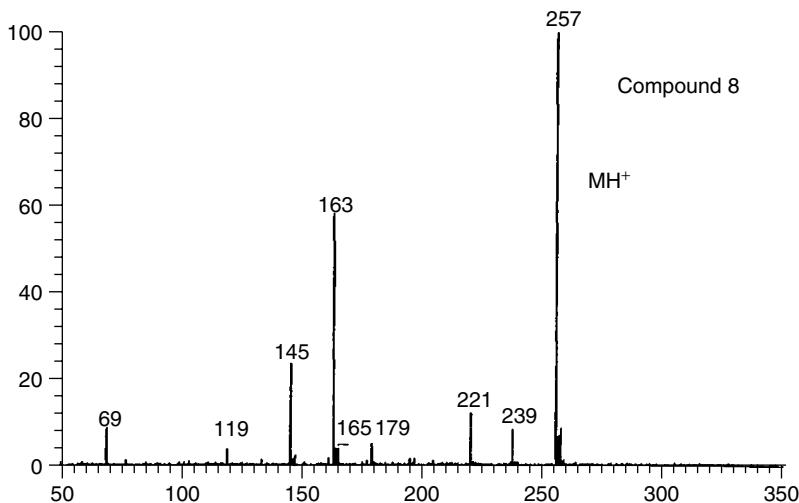
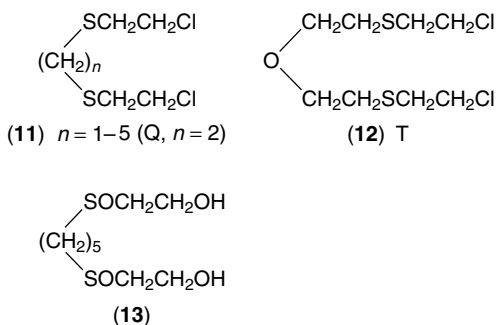


Figure 14. APCI mass spectrum of 1,5-bis(2-hydroxyethylsulfinyl)pentane

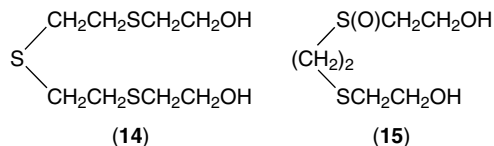
detection of one such compound (**13**) that was present in a proficiency test soil sample; Figure 14 shows the APCI mass spectrum of (**13**). LC/MS and NMR were the only techniques that reliably detected this analyte.



Hooijschuur *et al.* ⁽⁵⁴⁾ also reported the analysis of the hydrolysis products of sulfur mustard homologues (**11**), using micro-LC/ESI/MS (triple quadrupole) and micro-LC/FPD in sulfur mode. To improve sensitivity, large-volume injection was used with peak compression by adding suitable coeluting alcohols. LC employed a 0.28-mm ID packed C18 column, eluted isocratically with water-methanol (80:20 v/v)-0.2 % formic acid, flow rate 6 $\mu\text{L}/\text{min}$. Spectra were dominated by MH^+ , $[\text{MH} - \text{H}_2\text{O}]^+$,

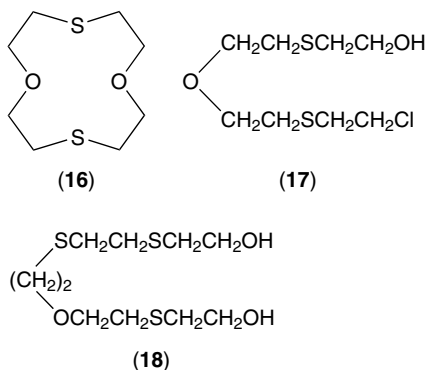
$[\text{MH} - \text{HOCH}_2\text{CH}_3]^+$ and $[\text{MH} - \text{HSCH}_2\text{CH}_2\text{OH}]^+$ ions; sodium adducts were also observed. Detection limits in full-scan mode were 0.5 $\mu\text{g}/\text{ml}$ for TDG, higher for the homologues. Kientz and coworkers ⁽⁸⁾ demonstrated the detection of TDG using an eluent jet interface. The EI mass spectrum showed a good match with a library EI spectrum.

LC/APCI/MS was applied to the analysis of aqueous extracts of soil samples collected from bomb craters associated with a CW attack on a Kurdish village. These samples had been previously analyzed by GC/MS and found to contain sulfur mustard and TDG. By searching the mass chromatograms of m/z 105 $[\text{HOCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2]^+$, two oligomeric products (**14**) and (**15**) were tentatively identified against a heavy background derived from the explosive tetryl. TDGO was readily detected by screening for the protonated molecule m/z 139.



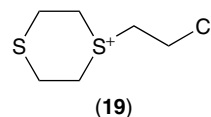
D'Agostino *et al.* ⁽⁵²⁾ used capillary LC/ESI/MS (conditions similar to those described in Section

6.3.1 for sarin and soman) to characterize a complex mixture of hydrolysis and other degradation products from three types of munition grade sulfur mustard. Samples of HD (= distilled mustard), HQ, which also contains Q, sesquimustard (**11**, $n = 2$), and HT, which also contains T (**12**), were hydrolyzed in water at 50 °C. Acetone was added to solubilize the remaining oil, excess water was removed, and the residues distilled. The samples were dissolved in water at 1 mg/ml before LC/ESI/MS analysis. ESI spectra were acquired on a hybrid magnetic sector-quadrupole instrument, at cone voltages that promoted CID; the resultant mass spectra exhibited molecular and fragment ions. Eleven compounds were identified, mostly linear and cyclic oligomers; examples are (**16**–**18**). Diol ESI/MS data were rich in molecular and fragment ion content, facilitating tentative structural identifications. Dimeric $[2M + H]^+$, $[M + Na]^+$, $[M + NH_4]^+$, MH^+ ions and fragment ions from loss of water were observed. Fragment ions resulting from sequential losses of SC_2H_4 or OC_2H_4 aided identification. Some of the higher mass diols were not detected during a previous analysis using GC/MS. LC/API/MS is particularly well suited to the analysis of the higher homologues because the presence of multiple heteroatoms renders these molecules thermally labile under GC conditions.



A problem with old mustard munitions is the presence of a solid or tarry residue, known as *heel*, which settles at the bottom of containers and may contain occluded mustard. This material is formed via sulfonium species. These cannot be detected by

GC/MS because of thermal lability, but their formation has been demonstrated by NMR. Rohrbaugh and Yang⁽⁵⁵⁾ investigated LC/ESI/MS for characterizing this material. To demonstrate a capability for detecting sulfonium species, six synthetic mustard-related cyclic and open-chain sulfonium species were analyzed. They were readily detected at a concentration of 0.01 M, with little or no fragmentation of the molecular ions. 1-(2-Chloroethyl)-1,4-dithianium (**19**) was identified as a major species in mustard heel (studies in the author's laboratory indicate much larger polymeric species are also present in heels). The methodology was further used to follow the hydrolysis of mustard and demonstrated the presence of the sulfonium species shown in Figure 12.

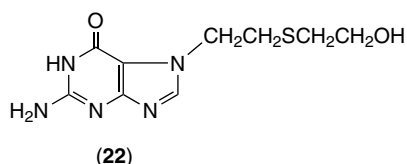
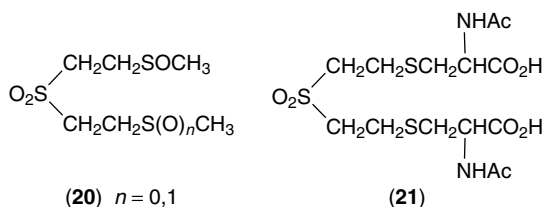


7.3 Biomedical Sample Analysis

7.3.1 Urinary Metabolites

LC/MS/MS is an important technique for the analysis of free metabolites and covalent adducts of sulfur mustard in urine and blood. In the case of TDG and TDGO, LC/MS has not yet been able to achieve the LODs obtainable with GC/MS after derivatization. LC/MS/MS has, however, been used successfully to analyze the metabolites (**20**, **21**) derived from an initial reaction of sulfur mustard with glutathione (see **Chapter 16**). The two metabolites (**20**), derived from the β -lyase pathway, can be isolated from urine by SPE on a hydroxylated polystyrene-divinylbenzene polymeric cartridge. Using a sensitive triple sector quadrupole LC/MS/MS system, detection limits of 0.1 ng/ml have been achieved using positive ESI and MRM⁽⁵⁶⁾. This provides a useful alternative to GC/MS/MS, which requires reduction of the sulfoxide functions with titanium trichloride. An LC/MS/MS method (detection limit 1 ng/ml) has been developed for the analysis of the bis(*N*-acetylcysteine) metabolite (**21**) in urine⁽⁵⁷⁾. Concentration from acidified urine was achieved on

a polymeric SPE cartridge and detection was by negative ion ESI using MRM. Attempts to analyze this metabolite by GC/MS after derivatization were unsuccessful and LC/MS/MS analysis of the dimethyl ester using thermospray ionization gave only a modest detection limit of 25 ng/ml, probably due to poor thermal stability in the thermospray ion source⁽⁵⁸⁾. Andersson *et al.*⁽⁵⁹⁾ characterized the initial bis-glutathione conjugate from which (**21**) is derived using LC/ESI/MS.



The N7-guanine adduct (**22**) is a urinary excretion product derived from the reaction of sulfur mustard with DNA. It can be isolated from urine by SPE on C18. GC/MS analysis of the derivatized adduct was problematic. A sensitive method was developed for the underivatized compound using LC/ESI/MS/MS, monitoring the fragmentation MH^+ , m/z 256 \rightarrow $[CH_2CH_2SCH_2CH_2OH]^+$, m/z 105 on a triple-sector quadrupole instrument⁽⁶⁰⁾. LC separation was on a C18 column eluted with water-acetonitrile-formic acid. The detection limit was 8 pg injected (S/N 5:1), 0.2 ng/ml in urine. Rao *et al.*⁽⁶¹⁾ also reported characterization by LC/ESI/MS but using 25 mM NH_4HCO_3 in 20 % MeOH as eluent.

7.3.2 Protein Adducts

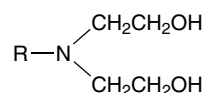
Sulfur mustard forms adducts with the blood proteins hemoglobin and albumin. Adducts with histidine residues are the most abundant after exposure of hemoglobin *in vitro* to sulfur mustard. Analysis of adducted histidine by GC/MS is hampered by poor thermal stability of volatile derivatives. A sensitive method was developed using LC/ESI/MS/MS after

derivatization to the *N*-9-fluorenylmethoxycarbonyl derivative^(62,63). Globin was hydrolyzed to its amino acid constituents in 6N HCl, the analyte concentrated by extraction on a cation exchange cartridge and derivatized with 9-fluorenylmethoxycarbonyl chloride. Recently, MALDI/TOF/MS of intact adducted hemoglobin was explored as a diagnostic tool for the confirmation of exposure to sulfur mustard⁽⁶⁴⁾; however, the methodology has not yet been reported for diagnostic purposes.

Noort *et al.*⁽⁶⁵⁾ showed that sulfur mustard alkylates a cysteine residue in human serum albumin. A sensitive method for detection of the adduct is based on Pronase digestion of alkylated albumin to the tripeptide *S*-[2-[(hydroxyethyl)thio]ethyl]-Cys-Pro-Phe, and detection using micro-LC/ESI/MS/MS.

8 NITROGEN MUSTARDS

The hydrolysis products (**23–25**) of the three nitrogen mustards listed in Schedule 1 of the CWC were included in the general LC/APCI/MS screening procedures of Black and Read^(14,26). These procedures, and those of other laboratories, have successfully identified *N*-methyldiethanolamine (**23**) and *N*-ethyldiethanolamine (**24**) in OPCW proficiency tests. More specific methods based on LC/ESI/MS have been reported for the trace analysis of ethanolamines in environmental residues⁽⁶⁶⁾ and biomedical samples⁽⁶⁷⁾.



- (23) R = Me (from HN-2)
 (24) R = Et (from HN-1)
 (25) R = HOCH₂CH₂ (from HN-3)

Alkanolamines are used during the 'sweetening process' in the oil and gas industry to remove toxic levels of gases such as hydrogen sulfide and carbon disulfide from raw gas condensates. Various alkanolamines such as *N*-methyldiethanolamine (**23**) and triethanolamine (**25**) may be utilized. They are therefore of concern as environmental contaminants of groundwater and wetland areas. Headley *et al.*⁽⁶⁶⁾

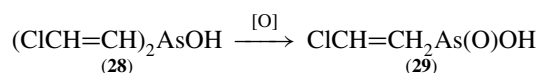
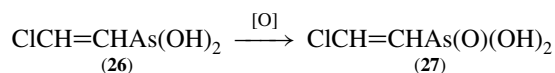
developed an LC/MS/MS method for the analysis of five ethanolamines, including (23) and (25), in vegetation. Filtered aqueous extracts, with added acetic acid to ensure the analytes were present in the protonated form, were chromatographed on a 250×2 -mm cation exchange column isocratically eluted with water-MeOH (1:1)-1 % formic acid, flow rate $200 \mu\text{l}/\text{min}$, split to deliver $30 \mu\text{l}/\text{min}$ to the ESI source. Using positive ESI and CID, the fragmentation $\text{MH}^+ \rightarrow [\text{MH} - \text{H}_2\text{O}]^+$ was monitored using a hybrid magnetic sector-quadrupole instrument. Detection limits were generally <20 ppb in vegetation. For CWC-related purposes, Lemire *et al.* (67) reported a similar method, but using a C18 LC column (150×2.1 mm) for the quantitative determination of *N*-ethyl- and *N*-methyldiethanolamine in urine. The analytes were concentrated from urine by SPE on a strong cation exchanger. In order to obtain good peak shapes on C18, 73 % 3 mM ammonium hydroxide (pH 10.5)-27 % methanol was used as the mobile phase for LC. Isotope dilution with $[\text{C}^{13}]_4$ -*N*-Me and *N*-Et diethanolamines was used to compensate for inherent variabilities. Detection was by MRM, monitoring the transition $\text{MH}^+ \rightarrow [\text{MH} - \text{H}_2\text{O}]^+$ using a triple sector quadrupole instrument. The limits of detection were 0.4 ng/ml for *N*-ethyldiethanolamine and 1 ng/ml for *N*-methyldiethanolamine. An interferent was present in urine with similar retention time and nominal mass characteristics as *N*-methyldiethanolamine.

Creasy (68) investigated postcolumn derivatization of *N*-ethyldiethanolamine and triethanolamine with benzoyl chloride in combination with APCI. Derivatization of one hydroxyl group occurred after mixing the reagent with LC eluent, and sensitivity was not affected by derivatization. A possible advantage of this method is to increase the molecular mass to remove the analyte from interferences.

9 LEWISITES

Lewisite is too reactive to be analyzed by LC. Even with GC, it leads to a rapid degradation of column performance. Lewisites 1 and 2 initially hydrolyze to 2-chlorovinylarsonous acid (CVAA) (26) and bis(2-chlorovinyl)arsenous acid (28), respectively. LC/MS analysis of these trivalent acids is problematic, giving very poor signal-to-noise ratios in both

ESI and APCI. LC/MS is considerably improved after precolumn oxidation with peroxide to the pentavalent acids (27) and (29). These can be chromatographed on a polymeric PRP-1 column using a standard water- CH_3CN -0.2 % formic acid gradient (69).

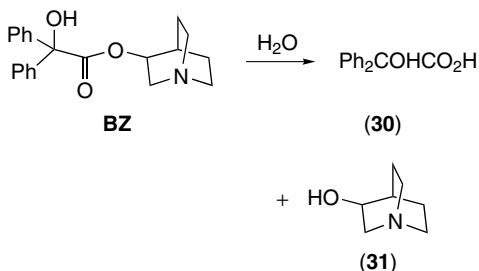


Alternatively, Creasy (68) used postcolumn derivatization of CVAA with 2-mercaptopyridine to improve the sensitivity (5–10 fold). CVAA appears to add a single molecule of the reagent to give a sulfonium species tentatively identified as $\text{ClCH}=\text{CHAs}=\text{S}^+\text{Py}$. Characterization of a munition grade lewisite mixture by micro-LC/MS/MS gave peaks that were difficult to interpret; two major components were tentatively identified as lewisite III oxide, $(\text{ClCH}=\text{CH})_3\text{AsO}$, and an oxidized lewisite II dimer (70). GC/MS methods employing derivatization with thiols and dithiols remain the methods of choice for the analysis of lewisites I and II and their degradation products, although they do not distinguish agent from hydrolysis product (18). In the case of CVAA, derivatization with thiols can be performed without the need to concentrate aqueous solutions to dryness.

10 3-QUINUCLIDINYL BENZILATE, BZ

No LC/MS studies targeted directly at BZ or its degradation products have been reported. Its hydrolysis products, benzoic acid (30) and 3-quinuclidinol (31), were included in the LC/APCI/MS screening procedures of Black and Read (14,26). BZ is also detected but with a long retention time. These procedures successfully identified BZ and 3-quinuclidinol in OPCW proficiency tests. The APCI spectra of BZ and 3-quinuclidinol are dominated by the MH^+ ions. MH^+ is not observed with benzoic acid, the base peak being m/z 211 $[\text{MH} - \text{H}_2\text{O}]^+$ with additional ions at m/z 228 $[\text{M} + \text{NH}_4 - \text{H}_2\text{O}]^+$, m/z 246 $[\text{M} + \text{NH}_4]^+$ and m/z 183. CID of m/z 211 gave m/z 183 ($-\text{CO}$) and 165 ($-\text{HCO}_2\text{H}$) (base

peak), 133 and 105. LC/MS/MS was poorly suited for confirming the identification of 3-quinuclidinol because relatively low intensity product ions were produced, even with a high-collision cell offset voltage. Benzoic acid gives a good response with both positive and negative ion APCI and ESI.



11 TOXINS

11.1 Overview

A major application of LC/ESI/MS is the characterization and detection of toxins, ranging from relatively small molecules, such as mycotoxins and some marine toxins, to the large proteinaceous toxins such as ricin and botulinum toxins. The marine toxin saxitoxin and the plant toxin ricin are specifically listed in Schedule 1 of the CWC as examples of toxins. A comprehensive review of LC/MS in toxin analysis would require a major chapter in its own right. Hancock and D'Agostino⁽⁷¹⁾ reviewed approaches to the mass spectrometric identification of selected low molecular mass toxins. This chapter will describe examples of LC/MS in the analysis of marine, fungal, bacterial, and plant toxins, which are of possible relevance to the CWC.

An important feature of ESI is the ability to characterize molecules with molecular mass up to ~300 kDa. Large molecules such as proteins are multiply charged with ESI, the charges being localized on the more basic amino acid residues such as lysine and arginine. The mass to charge ratios of these multiply charged molecules fall within the mass range of most analyzers. Along with MALDI/TOF, ESI has been the major mass spectrometric technique in characterizing proteins, carbohydrates, and other large biomolecules.

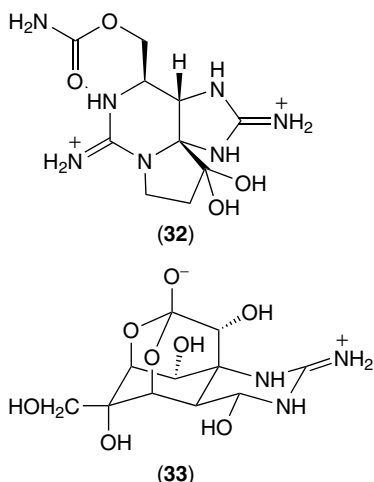
11.2 Criteria for Identification

The identification of toxins has not yet been addressed by the Technical Secretariat of the OPCW. One of the issues will be that identification for most toxins will only be possible using LC/MS and immunoassays. A working group of laboratories within NATO has organized analytical exercises for the identification of so-called mid spectrum agents (MSAs), which encompass toxins and peptide bioregulators. The group has developed criteria to define levels of confidence in an identification⁽⁷¹⁾. *Provisional identification* is based on either chromatographic retention data, acquired under two different experimental conditions, or the molecular mass, matching those of a known MSA, or alternatively a positive immunoassay. A *confirmed identification* is when any two of the above criteria are met or, in the case of proteinaceous MSAs, the molecular mass and mass map of peptides from enzymic digestion match those of a known MSA. Criteria for *unambiguous identification of low molecular mass, nonproteinaceous toxins* are similar to the OPCW criteria for chemicals, that is, identification by two spectrometric techniques, provided the molecular mass is confirmed. Finally, *unambiguous identification* of proteinaceous MSAs requires chromatographic data as defined for a provisional identification or a positive immunoassay, plus molecular mass and mass map data as defined for a confirmed identification, plus primary sequence data that matches that acquired from reference material under similar conditions.

11.3 Saxitoxin and Other Marine Toxins

Saxitoxin (32) is listed in Schedule 1 of the CWC. It is a polar, cationic, relatively low molecular mass toxin and is one of ~18 structurally related neurotoxins collectively known as *paralytic shellfish poisoning* (PSP) toxins. Analogues are formed by addition of sulfate, *N*-sulfo and *N*-hydroxyl groups, and by decarbamylation. They block neuronal sodium channels, and thereby neurotransmission, death resulting from respiratory paralysis. Saxitoxin is produced by dinoflagellate species (and by some freshwater cyanobacteria), and accumulates in shellfish. The cationic nature of saxitoxin makes capillary electrophoresis combined with

MS one of the methods of choice ⁽⁷²⁾. Analytical methods have focused mostly on generic detection of PSP toxins rather than saxitoxin alone ⁽⁷³⁾, and the different charged states of the related toxins is problematic with regard to finding suitable LC conditions. Positive ionspray is sensitive for saxitoxin (down to 30 pg injected under flow injection analysis (FIA) conditions using SIM) and gives an abundant $[M + H]^+$ ion ⁽⁷⁴⁾; positive ESI under nonoptimized FIA conditions was less sensitive (~ 800 pg injected) ⁽⁷⁵⁾. Pleasance *et al.* ⁽⁷²⁾ used a polymeric PRP-1 column, 200×1 mm, with a mobile phase of 10 mM aqueous ammonium formate modified with 5 % acetonitrile to demonstrate the LC/MS of saxitoxin and tetrodotoxin (**33**) (also a sodium channel blocker that accumulates in puffer fish). Using SIM, a LOD around $0.8\text{--}1 \mu\text{g/ml}$ was estimated for saxitoxin. For a better resolution of saxitoxin and its related toxins, a column of 150×4.1 mm was used with gradient elution with 5 mM ammonium formate-acetonitrile, flow rate 1 ml/min split 1:20. The toxins eluted in order of increasing positive charge, saxitoxin as a rather tailing peak with poor reproducibility.



Jaime *et al.* ⁽⁷⁶⁾ obtained superior chromatographic separation using a combination of anion and cation exchange columns connected in series (100 and 200×4.6 mm), and gradient elution with aqueous ammonium acetate (flow rate 0.8 ml/min, split 1:3 for MS); fluorescence detection using postcolumn

oxidation was also used. LODs for saxitoxin and its analogues were in the range $0.5\text{--}2$ ng/ml using ESI, and $0.01\text{--}0.8$ ng/ml using fluorescence detection. Lagos *et al.* ⁽⁷⁷⁾ detected saxitoxin and analogues in cultures of the freshwater cyanobacterium *Aphanizomenon flos-aquae* by LC/ESI/MS, using a 150×2 mm C18 column, with a mobile phase of 10 mM aqueous heptafluorobutyric acid (as ion pairing agent) plus 16 % acetonitrile, adjusted to pH 4.0 with NH_4OH .

11.4 Ricin

Ricin is a glycosylated proteinaceous toxin produced by the castor bean plant *Ricinus communis*. It is a potent inhibitor of protein synthesis, composed of two glycoprotein chains of 32 and 34 kDa, linked by a disulfide bridge. It is not a homogeneous toxin but exists as a series of glycosylated proteins, the composition of which depends on the particular cultivar, and there are other variants. Despeyroux *et al.* ⁽⁷⁸⁾ characterized ricin from a number of cultivars using loop injection in a mobile phase of water-acetonitrile, 1:1, plus 0.5 % formic acid. Data were recorded in the range m/z 975–1275. A mathematical deconvolution of the raw data provided mass profiles such as that shown in Figure 15. The pattern of glycosylation corresponded to additions of successive mannose residues. Darby *et al.* ⁽⁷⁹⁾ reported a method for the forensic detection of ricin based on LC/ESI/MS and MALDI/TOF/MS. The approximate molecular mass was determined by both techniques followed by characterization of tryptic digests by LC/ESI-ion trap MS. LC/ESI/MS used a 2.1×150 mm C18 column eluted with a gradient of water-acetonitrile-0.1 % TFA, interfaced to a quadrupole ion trap. MALDI/TOF was performed with a matrix of sinapinic acid in water-acetonitrile-0.1 % TFA. A series of noisy mass clusters was observed in the ESI spectrum, and these were only partially deconvoluted. MALDI/TOF gave a molecular mass peak with a centroid at m/z 62,766 but with an apex covering 300 mass units and a base width of ~ 4000 . More informative structural information was obtained from a tryptic digest. From a ricin standard, 11 peaks were detected by ESI and 14 by MALDI/TOF. Of these, only six were common to both techniques, illustrating the difference in selectivity. Fourteen of these tryptic peptides

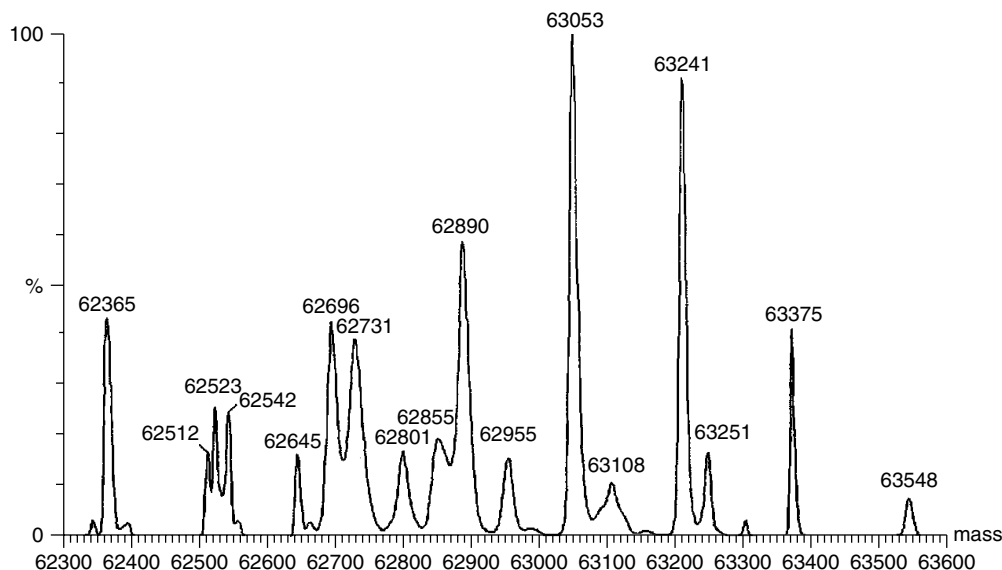


Figure 15. Deconvoluted ESI spectrum derived from the raw data for ricin. (Reprinted from *Analytical Biochemistry*, **279**, Despeyroux *et al.*, Characterization of Ricin Heterogeneity by Electrospray Mass Spectrometry, Capillary Electrophoresis, and Resonant Mirror, pp. 23–36 (2000), with permission from Elsevier)

could be matched to ricin-derived peptides in a database (MSFit) available on the Internet⁽⁸⁰⁾. Also identified was an alkaloid ricinine as a low molecular mass ($MH^+ = 165$) marker for the castor-oil bean; this could be analyzed by GC/MS or LC/MS.

11.5 Bacterial Toxins

Although none are specifically listed in the CW schedules, a number of bacterial toxins are of concern as chemical/biological agents (they are generally regarded as being covered by the Biological and Toxin Weapons Convention). The TNO group have characterized several of these toxins using micro-LC/ESI/MS. Kientz *et al.*⁽⁸¹⁾ determined the mass of Staphylococcal enterotoxin B (SEB) as $28,366.3 \pm 1.1$, using either flow injection analysis or micro-LC/MS using a gel phenyl column packing and a triple quadrupole MS. Additional characterization was obtained by detecting the presence of a disulfide bridge by the addition of 2-mercaptoethanol and by tryptic digestion. CID of the major tryptic fragments allowed the amino acid sequence of these peptides to be determined. It was noted that LC/MS/MS analysis of tryptic digests on micro C18 columns (600×0.3 mm), eluted with

a gradient of water-acetonitrile-0.5 % formic acid, resulted in a 30- to 40-fold increase in sensitivity compared to a conventional size column (250×5 mm). Cholera toxin, which comprises two noncovalently bonded units (A and B), was characterized similarly⁽⁸²⁾. Deconvolution of the ESI spectra gave average molecular masses of $27,210 \pm 0.8$ Da and $11,605.4 \pm 0.3$ for the A and B units, respectively.

Tetanus toxin, comprised of a light and heavy chain linked by a disulfide bridge (~ 52 kDa and ~ 98 kDa respectively) and botulinum toxins A and B, were similarly characterized using a quadrupole-TOF tandem MS^(83,84). Accurate masses of >50 tryptic digest fragments were determined in a single chromatographic run, illustrating the power of these instruments. Characterization was accomplished by accurate mass measurement of digest fragments, and amino acid sequencing of selected peptides using MS/MS. This procedure provided reliable identification data at levels above $1 \mu\text{g/ml}$.

11.6 Fungal Toxins (Mycotoxins)

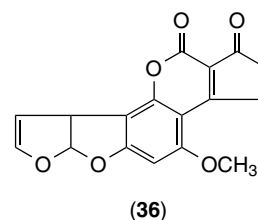
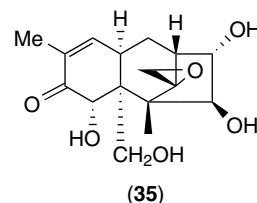
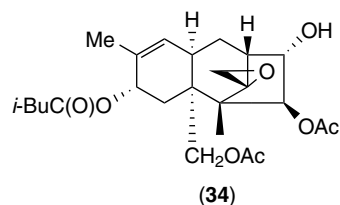
Two groups of mycotoxins have been of concern in a CBW context, trichothecenes produced by *Fusarium* species and aflatoxins produced by *Aspergillus*

species. Trichothecenes have been analyzed mainly by GC-based methods after derivatization, but a number of LC/MS methods have been reported. Both ESI and APCI have been used in positive and negative ion mode, depending on the analytes. Trichothecenes of the T-2 type (**34**) (type A) and nivalenol type (**35**) (type B) have been analyzed using positive ESI (ionspray)⁽⁸⁵⁾ and APCI^(86,87), using single and triple quadrupole instruments and ion traps. LC was performed on C18 columns with mobile phases of water-acetonitrile (\pm methanol)- NH_4OAc . APCI spectra showed strong ammonium adducts in a number of cases with extensive fragmentation. Trichothecenes of the nivalenol type contain an electrophilic enone system and are sensitive to negative ionization^(88,89). Nivalenol and deoxynivalenol were analyzed by negative APCI using a C18 column eluted isocratically with water-acetonitrile-methanol, 82:9:9⁽⁸⁸⁾, and by negative ESI using a gradient of water-methanol-0.3 % acetic acid⁽⁸⁹⁾. The latter study found negative ESI to be the more robust technique, particularly with regard to instrument fouling. Negative ESI spectra were substantially enhanced by the addition of acetic acid to the solvent, the spectra then being dominated by intense acetate adducts $[\text{M} - \text{H} + \text{AcOH}]^-$. The advantages of these methods are speed and simplicity, although lowest detection limits are probably still achieved with GC/MS.

Aflatoxins, for example, B_1 (**36**) and their biological markers have been determined using ESI^(90,91). Aflatoxins B_1 , B_2 , G_1 , G_2 were analyzed in food samples by LC/ESI/MS using a $150 \times 2\text{-mm}$ C18 column eluted isocratically with acetonitrile-MeOH-10 mM NH_4OAc (2:16:15)⁽⁹⁰⁾. The positive ESI spectra were dominated by the protonated molecules, which were used for SIM. The method enabled concentrations down to 1 ppb to be detected in various food materials. LC/ESI/MS/MS has been used for the detection of aflatoxin DNA adducts as urinary biomarkers of exposure⁽⁹¹⁾.

12 BIOLOGICALLY ACTIVE PEPTIDES

D'Agostino and coworkers⁽⁹²⁾ reported the analysis of several biologically active peptides, such



as substance P and bradykinins, using LC high-resolution ESI/MS. Multiply charged ions were observed and errors between observed and theoretical monoisotopic molecular masses were typically in the 5–30 ppm range during LC/ESI/MS at magnetic sector resolutions between 2500 and 6000. CID generated a series of sequence ions enabling the amino acid sequences to be determined. The methodology was successfully applied to the identification of five unknown peptides in an international round robin analytical exercise.

13 LC/MS AS A COMPONENT OF MULTIPLE ANALYTICAL TECHNIQUES

LC/MS is an important addition to the techniques used routinely to analyze unknown samples. It is not a substitute for other techniques but is complementary, for example, to GC/MS, GC/FTIR, and NMR. In OPCW proficiency tests, a number of laboratories use single-stage LC/MS as a rapid screening procedure for water samples and aqueous extracts of matrices such as soil, and GC/FPD or

GC/MS for screening organic solutions and extracts. LC/MS usually provides molecular mass data, which may be used as a second technique in support of GC/EI/MS data. Varying degrees of structural information can be obtained from LC/MS/MS. If analyte concentration permits, and the sample is relatively clean, NMR can provide valuable complementary structural information, particularly in differentiating structural isomers. The combination of LC with NMR, which currently is very expensive, will eventually provide a very powerful technique to complement LC/MS. The successful use of FIA/ESI/MS during a proficiency test is described by Hooijschuur *et al.* ⁽²¹⁾ (this laboratory also used micro-LC/FPD for screening). The authors' laboratory applied LC/APCI/MS as a screening procedure in the same proficiency test. Positive LC/APCI/MS rapidly detected analytes in a water sample with protonated molecules at m/z 120 and 134, tentatively identified as *N*-methyl and *N*-ethyldiethanolamine. Ethylphosphonic acid was detected, albeit rather weakly, in positive and negative ion mode. Screening of an aqueous extract of a soil sample detected five analytes not present in the blank sample, the four reportable ones being tentatively identified as TDGO, methyl EPA, dimethyl ethylphosphonate and 1,5-bis(2-hydroxyethylsulfinyl)pentane. With the first three compounds, unequivocal identification to OPCW standards was achieved in the author's laboratory using a combination of LC/MS and GC/MS. In the case of 1,5-bis(2-hydroxyethylsulfinyl)-*n*-pentane, detection and characterization by GC/MS after derivatization was difficult because of thermal instability, and a combination of LC/MS, LC/MS/MS and NMR was used for confirmation (see Figure 13).

LC/MS is particularly useful in combination with other analytical techniques in decontamination/demilitarization studies. Creasy *et al.* ⁽⁹³⁾ used multiple techniques to characterize decontamination waste from nerve agents and sulfur mustard at a former CW storage site. LC/MS was particularly useful in analyzing waste from VX, and in characterizing a very complex mixture of products from the hydrolytic/oxidative decontamination of sulfur mustard. Positive APCI identified a range of products such as TDGO and cyclic sulfoxides, and negative APCI identified a number of hydroxylated and chlorinated alkylsulfonic acids. The same group characterized a complex mixture from a munition

containing a CW simulant using GC/MS, GC/IR, LC/MS, and NMR as the primary techniques ⁽⁹⁴⁾.

14 CONCLUSIONS

LC/MS has become a widely used and robust analytical technique. Its overwhelming advantages are its applicability to a broad range of analytes (particularly with ESI) and its direct applicability to aqueous samples. Other than for trace analysis, it should be the method of choice for analyzing polar degradation products in water samples, decontamination solutions, and aqueous extracts of matrices such as soil. LC/MS provides a very rapid and effective screening and identification procedure in OPCW proficiency tests, which complements methods based on GC and NMR. LC/MS/MS plays a key role in biomedical sample analysis. In many cases, LC/MS is the only technique suitable for the unequivocal detection of adducts of CW agents with macromolecules, as biological markers of exposure. It is also being used increasingly for the trace analysis of free metabolites in urine and blood, as the latest generation instruments compete with GC/MS in terms of sensitivity. It is important that LC/MS is not regarded as a substitute for GC/MS but as a complementary technique. The combination of LC/MS, GC/MS, GC/FTIR, GC with selective detectors, and NMR provides a battery of techniques that should be capable of detecting and identifying any organic material of relevance to the CWC.

ABBREVIATIONS AND ACRONYMS

APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric Pressure Ionization
APPI	Atmospheric Pressure Photo-Ionization
BSTFA	Bis(Trimethylsilyl)Trifluoroacetamide
CID	Collision Induced Dissociation
CVAA	Chlorovinylarsonous Acid
CW	Chemical Weapons or Chemical Warfare
CWC	Chemical Weapons Convention
DMMP	Dimethyl Methylphosphonate
EI	Electron Ionization

EIC	Extracted Ion Chromatogram
EMPA	Ethyl Methylphosphonic Acids
EPA	Ethyl Phosphoric Acid
ESI	Electrospray Ionization
ESI-MS	Electrospray Ionization/Mass Spectrometry
FIA	Flow Injection Analysis
FTICR	Fourier Transform Ion Cyclotron Resonance
GC/MS	Gas Chromatography/Mass Spectrometry
HFBA	Heptafluorobutyric Acid
LC	Liquid Chromatography
LC/MS	Liquid Chromatography Combined With Mass Spectrometry
LOD	Limits of Detection
MPA	Methylphosphonic Acids
MRM	Multiple Reaction Monitoring
MSA	Mid Spectrum Agents
MS/MS	Tandem Mass Spectrometry
OPCW	Organization for the Prohibition of Chemical Weapons
PGC	Porous Graphitic Carbon
PSP	Paralytic Shellfish Poisoning
RIC	Reconstructed Ion Chromatogram
SEB	Staphylococcal Enterotoxin B
SIM	Selected Ion Monitoring
SPE	Solid-Phase Extraction
TDG	Thiodiglycol
TDGO	Thiodiglycol Sulfoxide
TEAH	Tetraethylammonium Hydroxide
TFA	Trifluoroacetic Acid
TIC	Total Ion Chromatogram
TOF	Time of Flight

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CHAPTER 13

Nuclear Magnetic Resonance Spectroscopy in Analysis of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful analytical techniques in organic chemistry for elucidating the molecular structures of chemicals ^(1,2). Moreover, an NMR spectrum may be used like a fingerprint to identify a chemical by comparing it with its reference spectrum recorded from the authentic chemical under comparable conditions. The spectrum also reveals information on molecular conformation, isomerism, molecular dynamics, and diastereomers ⁽³⁻⁶⁾.

During several international interlaboratory comparisons (round-robin) and proficiency tests for the

verification of chemical disarmament, NMR spectroscopy proved itself to be a useful complementary technique to mass spectrometry (MS) and infrared (IR) spectroscopy ⁽⁷⁻¹⁵⁾. Test samples included aqueous and organic liquids, soils, sands, concrete, paints, and rubbers spiked with chemicals related to the Chemical Weapons Convention (CWC), usually at levels of about 10 ppm ⁽¹⁶⁾. NMR has been applied for related field sample analysis as well ^(17,18).

NMR spectroscopy is applicable to all chemicals dissolving in sufficient amount in deuterated solvent. Nondeuterated solvents can be used if the experiment is performed unlocked or if a small amount of the corresponding deuterated solvent is added to

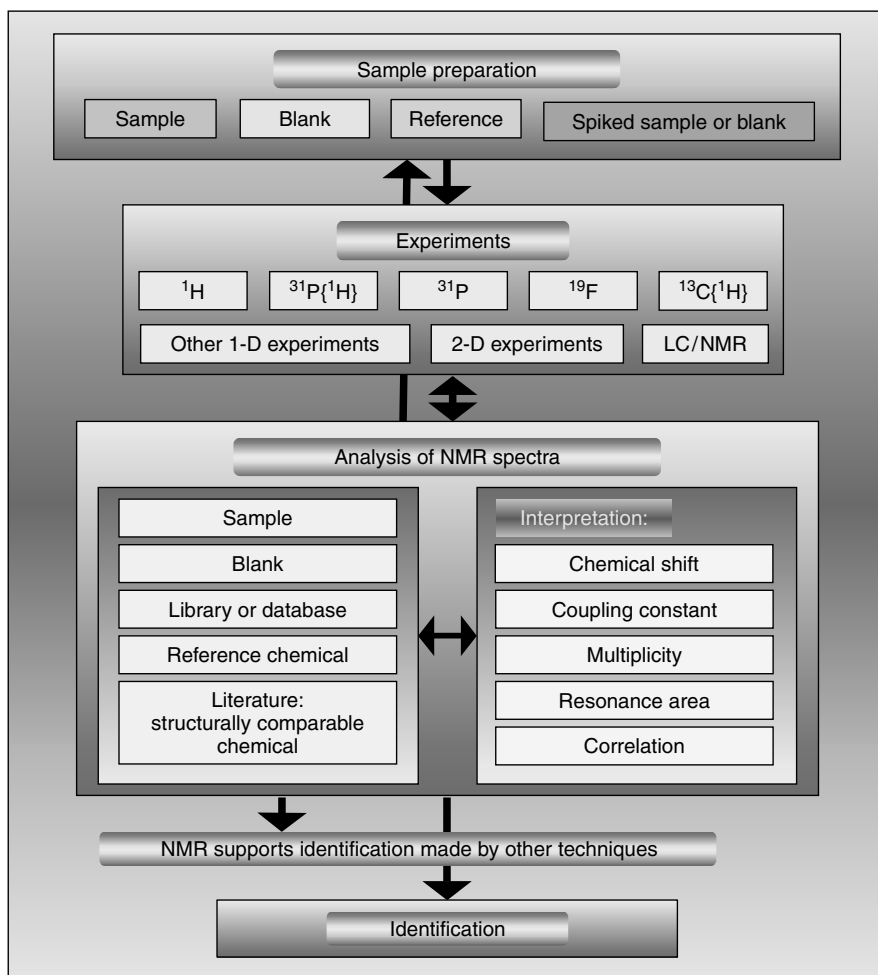


Figure 1. Verification of CWC-related chemicals by NMR spectroscopy

provide the lock conditions. This article describes the NMR spectroscopic methods used in the analysis of Chemical Warfare (CW) agents and related chemicals. The flow chart shown in Figure 1 demonstrates the application of NMR spectroscopy to the field of verification of CWC-related chemicals.

The most common nuclei observed are ^1H , ^{31}P , ^{19}F , and ^{13}C . For high concentrations (>1000 ppm) of CWC-related chemicals and low background levels, the ^1H , $^{13}\text{C}\{^1\text{H}\}$, ^{19}F , $^{31}\text{P}\{^1\text{H}\}$, and ^{31}P NMR experiments are all useful for identification. For the analysis of chemicals present in trace amounts (low ppm level; in this text, for example, concentration of 1 ppm is equivalent to $1\text{ }\mu\text{g}$ of chemical in 1 mL (or 1 g) of sample) in environmental samples, the more sensitive ^1H NMR experiment is the method of choice. A 400-MHz spectrometer will generally offer sufficient sensitivity for the detection of chemicals present in trace amounts, although unknown amounts of background may cause problems. The ^{19}F and $^{31}\text{P}\{^1\text{H}\}$ experiments are useful as screening techniques to check for the presence of these nuclei. The acquisition of proton-coupled ^{31}P spectra, which reveal more detailed structural information, is limited more by the poor sensitivity than by background problems. Recording of $^{13}\text{C}\{^1\text{H}\}$ NMR spectra may be futile in concentrations below 100 ppm. In the international tests, the low sensitivity of NMR experiments was compensated in part by concentrating the NMR samples and by running long-term experiments.

Compared with MS and IR spectroscopy, the main problems in NMR experiments are the lower sensitivity and the resonance overlapping caused by large amounts of background chemicals present in the sample. Techniques other than NMR are usually hyphenated with gas chromatography (GC) or liquid chromatography (LC) to provide the superior analytical power achieved by combining selectivity and sensitivity. However, the structural specificity of NMR spectra, the capability for the observation of different nuclei, and the 2-D correlation experiments make NMR spectroscopy a valuable technique to be used alongside other techniques. With regard to the chemicals listed in the Schedules of the CWC ⁽¹⁶⁾ (see **Chapter 2**), NMR spectroscopy is particularly useful for the analysis of alkylphosphonates (e.g. precursors or degradation products of nerve agents), thioalcohols, and aminoalcohols and their salts, but it is not limited to these.

2 INSTRUMENTS AND EQUIPMENT

2.1 Spectrometer

The following are recommendations for an NMR spectrometer suitable for the analysis of CWC-related chemicals:

- capability for observing ^1H , ^{13}C , ^{19}F , and ^{31}P nuclei (recording ^1H , $^{13}\text{C}\{^1\text{H}\}$, ^{19}F , $^{31}\text{P}\{^1\text{H}\}$, and ^{31}P NMR spectra);
- a proton resonance frequency preferably of 200 MHz or more;
- capability for control of the sample temperature to an accuracy of within $\pm 0.5^\circ\text{C}$; and
- a data system adequate for acquisition, processing, printing, and storage of all necessary data ⁽¹⁹⁾.

Modern computerized high-field NMR spectrometers normally fulfill these requirements and, in addition, are routinely capable of running the latest pulse sequences to record the one- and two-dimensional (1-D and 2-D) spectra useful for structure elucidation of unknown chemicals. The higher the resonance frequency, the smaller is the amount of a chemical needed for its identification. Higher-frequency instruments also provide larger resonance dispersion, which simplifies the spectra and helps in their analysis. In the interlaboratory comparison/proficiency tests, the proton frequencies have ranged from 250 to 600 MHz, but even the lower-frequency instruments have produced useful spectra. Temperature control is recommended because line broadening may occur with a drift in temperature – an effect that may be particularly important during long-term accumulations.

2.2 Probe Heads

The capability of recording the spectra of different nuclei depends on the probe head. ^1H , ^{13}C , ^{19}F , and ^{31}P nuclei are observed (in some experiments decoupled) in verifying CWC-related chemicals, and the probe head is selected accordingly. The most common probe head diameters are 5 and 10 mm. For the same sample concentration, a 10-mm probe head is more sensitive than a 5-mm probe head, but the former requires a larger sample volume and

accordingly more analyte. The 5-mm probe heads were the most common choice in the interlaboratory comparison/proficiency tests; in the selection of a probe head, its sensitivity for a particular nucleus is also important. For probe heads available, see catalogues of NMR spectrometer and probe head manufacturers.

The most common probe head is a switchable probe head, which can be used to observe ^1H and all NMR-active nuclei from the low-frequency limit up to the frequency of ^{31}P . The proton coil can be tuned for the observation of ^{19}F . The switchable probe head is designed for either direct or inverse observation. The direct observation probe head is most sensitive for 1-D experiments on ^{13}C and ^{31}P . The inverse probe head in turn is most sensitive for the direct observation of ^1H and indirect detection, for example of ^{31}P , in 2-D experiments, taking advantage of polarization-transfer phenomena.

A four-nucleus probe head with which ^1H , ^{13}C , ^{19}F , and ^{31}P can be observed is also useful. Such a probe head can be pretuned for each nucleus, and the switching between nuclei is easy and can even be automated. Sensitivity is comparable to that of the switchable probe head with direct observation.

Selective probe heads are used for optimal sensitivity for a particular nucleus. Sensitivity of a selective ^1H probe head is normally greater than that of a switchable probe head with indirect observation. With a selective X-nucleus (a nucleus other than proton) probe head, decoupling of protons is normally possible. Because of their limited usefulness, selective probe heads are rare in NMR laboratories. Other probe heads are also available, for example, those for triple resonance experiments and experiments utilizing pulsed-field gradients. In addition to their suitability for 2-D experiments, the gradients are particularly suitable for solvent suppression ⁽²⁰⁾.

2.3 Automatic Operation

An automatic sample changer (ASC) often is an option for a spectrometer. The operator sets the samples into the rack of the ASC and predefines the experiments, which are then performed with automatic homogeneity adjustment, processing,

and plotting of data. When the experiments for one NMR sample are completed, the sample is changed. In this way, the spectrometer can work unattended during nights and weekends. The ASC works best for recording spectra from standard NMR samples in which the concentration is relatively high; also, the possibly failed experiments then can be directly rerun. However, during the testing, the time is limited and repetition of an experiment, which lasted, for example, for 16 hours may be impossible. Requirements for optimal experimental performance for measuring test samples, which may contain anything from organic liquids to aqueous liquids of high salt content, may be too demanding for the ASC.

The main requirements for measuring test samples are (a) separate tuning of the probe head for each sample and nucleus in order to guarantee optimum sensitivity, (b) accurate homogeneity adjustment even for the most difficult samples, in order to achieve the narrowest lines and pure line shapes, and (c) plotting of expanded regions for spectra where the resonances of interest may be less than 1 % of the intensity of the background resonances. Manual operation by an experienced operator is mandatory if highest-quality spectra are to be guaranteed.

Automatic operation is possible without a sample changer if 'macros' or programmable spectrometer software is available. After manual tuning of the observed nucleus, manual homogeneity adjustment, and a manual check for an acceptable line shape, defined experiments can be run one after the other with a single sample. A series of experiments can be run overnight or longer, with the computer taking care of the measurements. The data are plotted manually for evaluation and analysis.

The spectral analysis is carried out manually because automatic interpretation and library programs are normally not available. Difficulties in NMR and automatic interpretation are (a) high spectral background in spectra recorded from environmental samples, often leading to resonance overlap, (b) solvent dependence of chemical shifts (δ), which with couplings affects the appearance of the spectrum, and (c) in the case of ^1H NMR spectra, the complexity. The other spectra, particularly $^{13}\text{C}\{^1\text{H}\}$, are simple, but low sensitivity is then a problem.

2.4 Data Systems

The spectrometer computer is equipped with an operating system (e.g. a version of UNIX, Windows, or Linux) and the specific operating system for the spectrometer. After the experiments, data can be stored on different media, sent through the Internet, processed in various ways, and plotted.

3 EXPERIMENTAL

3.1 Solvents

Deuterated solvents are preferred for NMR samples. The solvents should be of spectroscopic grade or corresponding quality and should have a high enrichment of deuterium, preferably >99.5 %. Deuterium oxide (D_2O), chloroform- d_1 ($CDCl_3$), acetone- d_6 ($(CD_3)_2CO$), and dichloromethane- d_2 (CD_2Cl_2) are common in the analysis of CWC-related chemicals, but many others (e.g. acetonitrile- d_4 (CD_3CN), methanol- d_4 (CD_3OD)) are available and may be suitable. The solvent is selected according to the requirements of the sample preparation. Where evaporation of the (protonated) solvent is not desirable, a small portion of the corresponding deuterated solvent is added to the sample (e.g. 5–10 % v/v) for the field/frequency stabilization.

The impurities in some solvents may disturb the analysis of low ppm concentrations of CWC-related chemicals through resonance overlap. Traces of water are common in organic solvents, but the water can be removed by using a suitable drying agent in contact with the sample solution before transferring it to an NMR tube. A representative blank sample, if available, or a blind sample (solvents and reagents that have gone through the sample preparation path without having been in touch with the test sample) may serve for excluding the resonances of the background and solvent chemicals from the spectrum of the test sample.

3.2 Chemical Shift References

Chemical shift references for calibration of the spectrum scale may be internal or external. An internal chemical shift reference substance is dissolved

directly in the NMR sample solution that is measured. In the external method, a separate NMR sample is prepared, containing the reference substance(s) dissolved in the same solvent as used for the test sample. Alternatively, the reference substance may be placed in a coaxial insert (capillary) tube, as such (e.g. 85 % H_3PO_4) or dissolved in the same solvent as used for the test sample. The insert tube (e.g. Wilmad WGS-5BL or equivalent) is placed into the NMR sample to be analyzed, or into a separate NMR tube (e.g. Wilmad 507-PP-7 or equivalent) containing only the solvent. The spectrum is then recorded for the reference position.

To avoid overlap of the resonances of the reference substance and chemicals of interest, and a possible overdose of the reference substance, and also from the point of view of optimum magnet homogeneity, we recommend the external chemical shift reference method with the use of a separate sample tube.

The external reference method is common in ^{31}P NMR. The reference substance, 85 % H_3PO_4 (phosphoric acid), is transferred from a cylindrical or spherical capillary tube to an NMR sample tube containing the same solvent as the test sample. Owing to its chemical reactivity, H_3PO_4 cannot be added as an internal reference substance. Although not an exact method ⁽²¹⁾, the resonance of H_3PO_4 is set at 0.00 ppm when the coaxial capillary tube system is used.

The most common reference chemicals in 1H and ^{13}C NMR are tetramethylsilane (TMS; dissolves in organic solvents, $\delta_H = \delta_C = 0.00$ ppm), 3-(trimethylsilyl)-3,3,2-tetradeuteriopropionic acid sodium salt (TSPA- d_4 ; dissolves in water/ D_2O , $\delta_H = \delta_C = 0.0$ ppm), and 3-(trimethylsilyl)propane sulfonic acid sodium salt (TSPSA; dissolves in water/ D_2O , $\delta_H = 0.015$ ppm, $\delta_C = 0.00$ ppm) ⁽²²⁾. A common reference substance for ^{19}F NMR is trichlorofluoromethane ($CFCl_3$, dissolves in organic solvents, $\delta_F = 0.00$ ppm). The fluorine resonance of $CFCl_3$ consists of four lines with intensities (from high-frequency down) 8:8:3:1; the second line from the high-frequency end is used as the scale origin ⁽²³⁾.

Other reference substances are available and can be used as well. Sometimes the resonance of the actual solvent can serve as a reference. Preferably, the referencing method used for the sample should be the same as that used for the authentic reference sample (or library spectrum), or at least the scale

position should be corrected by a known factor. Whatever referencing method is used, it needs to be reported in sufficient detail.

3.3 Nuclear Magnetic Resonance Sample Tube

NMR tubes are usually made of high-quality Pyrex glass. The tube is selected according to the size of the probe head, normally 5 or 10 mm (o.d.). Different qualities of tubes are available. Provided the sample solution is of good-quality, thin-walled extra-high-quality NMR tubes give best resolution, line shape, and sensitivity. Routine quality tubes for daily use (e.g. Wilmad 507-PP or equivalent) are normally adequate, however. Usually the tube is sealed by a plastic cap, but other cap materials are also available. For the sake of safety in working with toxic chemicals, sealing of the tube by melting the top of it by flame may be advisable (an air-tight ampoule is formed). Microcylinders and coaxial capillary inserts of different shapes, volumes, and materials are available for small sample amounts, external locking, external referencing, and corrosive materials (see catalogues of NMR tube sellers). By way of example, good results from analysis of low-volume, low-concentration samples have been obtained using Shigemitsu tubes ⁽²⁴⁾.

A simple method to remove dust, which even new NMR tubes may contain, employs a 10–20-mL plastic syringe, a 0.45- μ m high-performance liquid chromatography (HPLC) filter unit suitable for water, a needle, distilled water, and an oven (at +45–55°C). Rinse the NMR tube with 1-mL portions of filtered distilled water by shaking while the cap is on, until no dust is seen in the rinsing solution in the tube. Empty the NMR tube and put it open-end down in a decanter glass, and into the oven for drying. Do not let the tubes stand in the warm oven longer than necessary for drying.

3.4 Sample Preparation

In principle, preparation of an NMR sample is simple: take a suitable amount (1–100 mg) of chemical to be analyzed, dissolve it in a selected solvent, add the chemical shift reference, and then transfer the sample with simultaneous filtration into an NMR

tube. Attach a code label to the top of the NMR tube. General reading on NMR sample preparation is available ^(25–27).

The quality of the results in NMR largely depends on the care with which the sample has been prepared. Not only should the NMR sample tube be of good (routine) quality but also the sample solution in the tube should be free of nondissolved particles and dust.

NMR spectral parameters, that is, chemical shift (δ) and coupling constant (J), may be considerably affected by the sample condition, that is, solvent, pH, sample temperature, concentration, and choice of internal and/or external chemical shift references. Solvent and pH (in water/D₂O samples) have the greatest effect. The sample condition should therefore be the same as or comparable to that used for the authentic reference chemical (or library spectrum) and the blank sample.

A sample for NMR spectroscopy can be taken from several stages of the sample preparation path (see **Chapter 9**) ^(14,19,28). Preferably, this would be a 5–10-mL portion of extract or of aqueous or organic liquid. Common to all these solutions is the large molar excess of ¹H in the solvent compared to the amount of ¹H in the possible target chemicals. This yields an intense solvent (e.g. H₂O, CH₂Cl₂) resonance in the ¹H NMR spectrum, making the trace analysis difficult or even impossible. A usual procedure to avoid this problem is to replace the protonated solvent (e.g. H₂O) with the corresponding deuterated solvent (D₂O). Deuterated solvents are also used for the field-frequency lock of the spectrometer.

The solvent is replaced by evaporating the protonated solvent and adding the corresponding deuterated solvent in its place. The sample is concentrated during the evaporation; however, one must be alert to the danger of losing volatile chemicals at the same time. The sample solution is then transferred to an NMR tube and is ready for the experiments. Because NMR spectroscopy is a nondestructive technique, the sample can be used after the NMR experiments for other analyses.

Except for extraction, no separation methods have been used in the NMR sample preparations in the interlaboratory comparison tests ^(7–15). Thus, the chemicals of interest, impurities, and background present in the original sample and in the extract were all present in the NMR sample. The advantage of this

is that, generally, not much or nothing is lost, while the disadvantage is overlapping of the resonances of interest with resonances of other chemicals.

3.4.1 Preparation of NMR Samples from Aqueous or Organic Liquid or Extract

A portion of aqueous or organic liquid or extract reserved for NMR spectroscopy is filtered, if necessary, and concentrated by evaporation. The evaporation is carried out on a rotary evaporator ($+50^{\circ}\text{C}$, 35 mmHg for evaporation of water), or by a gentle flow of nitrogen gas (for organic liquid) with warming ($40\text{--}50^{\circ}\text{C}$) or without. The small amount (ca. 0.5–1 mL) of solution left in the rotary evaporator is further evaporated nearly to dryness by a flow of nitrogen gas. To each concentrate, about 1 mL of the corresponding deuterated solvent (e.g. D_2O , CD_2Cl_2) is added and the solution is again evaporated nearly to dryness. These two steps are then repeated. The remaining solution is filtered through a cotton plug (or a plug of glass wool) placed in the neck of a Pasteur pipette into a washed, dust-free NMR tube.

Alternatively, an HPLC filter unit may be used for the filtration. The sample vessel is rinsed with a suitable amount of (deuterated) solvent and the rinsing solutions are added to the sample in the NMR tube. The pH of the aqueous liquid sample is determined, and can be adjusted with DCl and NaOD solution. For measurements carried out in a standard 5-mm (o.d.) NMR tube, about 0.6–0.8 mL of solvent is required, and for 10-mm tubes, about 3–4 mL. If considered necessary, some of the extractions can be performed with deuterated solvents, which will simplify the sample preparation procedure. Related practical sample preparations can be found in the literature ^(10,12,14,29).

Because chemicals that distill easily or with water (e.g. pinacolyl alcohol) may be lost during rotary evaporation, an additional sample may be prepared for screening/analysis by taking a suitable amount of original aqueous liquid (e.g. 0.7 mL for a 5-mm o.d. tube), adding a small amount (e.g. 0.1 mL) of D_2O , determining the pH, and filtering the sample solution into an NMR tube. A similar nonevaporated NMR sample can be prepared from the organic extract. Note that although it is important to know the pH of aqueous NMR sample, its determination may be left after the NMR experiments; in this

way, the precious sample remains untouched until the experiments have been finished.

If an internal chemical shift reference is to be used, a dilute solution of reference substance (e.g. TSPA- d_4) is prepared in the same solvent as used for the test sample (e.g. D_2O). A few microliters (or a suitable amount) of this reference solution are added to the evaporated sample solution before filtration into the NMR tube.

3.4.2 Preparation of NMR Samples from Authentic Reference Chemicals

In a 2-mL vial, dissolve the selected amount of authentic reference chemical (e.g. 20 μL of mustard gas (HD)) in 0.8 mL of deuterated solvent (e.g. CDCl_3). (To ensure safety, always prepare NMR samples of toxic chemicals in a properly equipped fume cupboard or glove box.) Using a Pasteur pipette, add as an internal chemical shift reference three to four drops of TMS solution, prepared by dissolving three to four drops of TMS in 0.5 mL of solvent (CDCl_3 in this case). Place a plug of purified cotton for a filter tightly in the neck of a Pasteur pipette, and fix the pipette on a clamp stand. Rinse the cotton plug with the sample solution, then let the solution flow into the washed, dust-free NMR tube. Seal the tube with a plastic cap and parafilm or with a suitable vacuum and welding apparatus, flame seal the tube near its open end in a low vacuum. It should be noted that some organic solvents evaporate through the plastic cap used in NMR tubes.

Alternatively, instead of cotton, glass wool or a suitable HPLC filter unit may be used for the filtration. Before filtering the authentic reference sample solution (in D_2O) into the NMR tube, adjust the pH to the same value as in the test sample.

See Figures 2 and 3 for NMR spectra of authentic sarin (GB) and tabun (GA) and Table 1 for NMR spectral parameters and sample conditions for some other authentic samples.

3.5 Experiments and Data Processing

3.5.1 Preliminary Preparation

The probe head is separately tuned and matched ⁽²⁵⁾ for each sample and experiment. The magnetic field

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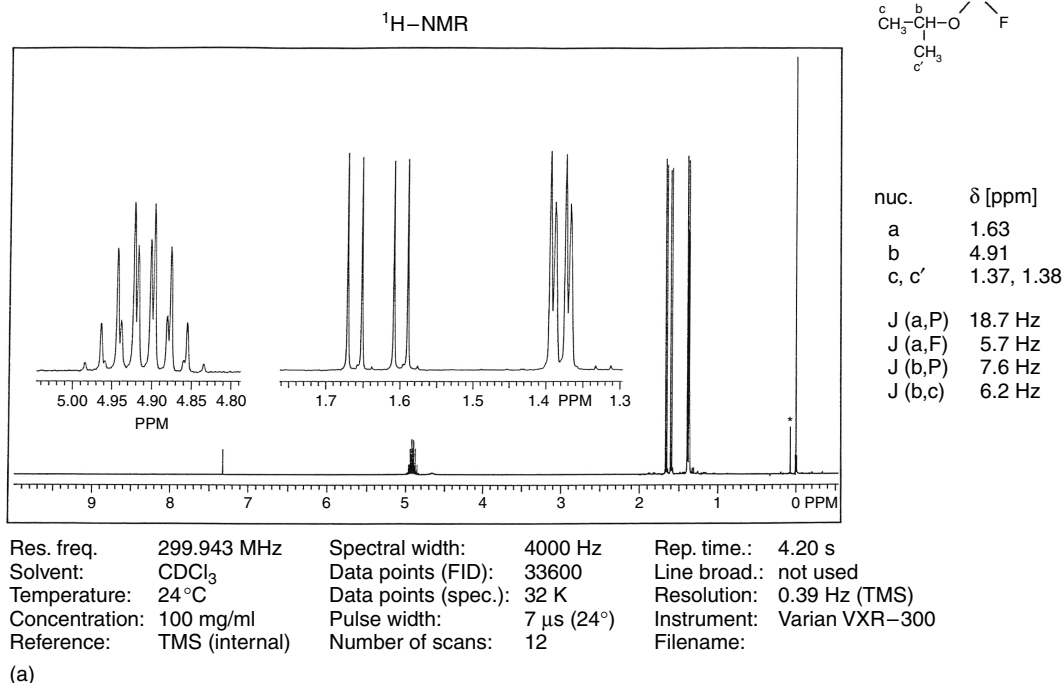


Figure 2. (a) ^1H , (b) $^{31}\text{P}\{^1\text{H}\}$, (c) ^{31}P , (d) ^{19}F , and (e) $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of sarin (GB) in CDCl_3 from the Atlas of NMR Spectra of Spiez Laboratory

homogeneity is carefully adjusted⁽²⁵⁾ and, always before final accumulation, line shape and line width are checked by recording trial 1-D spectra with a few scans (usually ^1H NMR spectrum serves well in checking). Every experiment run in the laboratory should be logged in an experiment logbook; a useful reference entry would contain a run number, the date, the file name, the sample code, and the initials of the operator.

3.5.2 Experimental Conditions

Unlike the sample condition, the experimental parameters have only a minor effect on the NMR spectral parameters. Experimental parameters such as spectral width, flip angle, repetition time, number of points in the free induction decay (FID) and in the real spectrum, number of scans, and processing parameters need to be comparable to those used for the acquisition of the database spectrum or spectrum of the authentic

reference chemical. The experimental parameters determine whether quantitative information can be obtained from an NMR spectrum: under certain experimental conditions, the resonance area is directly proportional to the number of nuclei⁽³⁰⁾.

^1H and ^{19}F NMR spectra are recorded with a normal one-pulse sequence or, alternatively, the ^1H spectra are recorded with a sequence that allows simultaneous solvent suppression with presaturation⁽³¹⁾ or a sequence that includes some other method of suppression; $^{13}\text{C}\{^1\text{H}\}$ and $^1\text{P}\{^1\text{H}\}$ spectra are recorded with proton broadband (composite pulse) decoupling⁽³²⁾, and ^{31}P spectra with gated proton decoupling⁽³³⁾.

The experimental conditions are the following: spectral ranges (all approximate) are from -0.5 to 11 ppm in ^1H NMR, from -40 to 120 ppm in $^{31}\text{P}\{^1\text{H}\}$ and ^{31}P NMR (certain types of phosphorus-containing chemicals, e.g. phosphonites and phosphites, may have larger chemical shifts and their observation needs an extended spectral window),

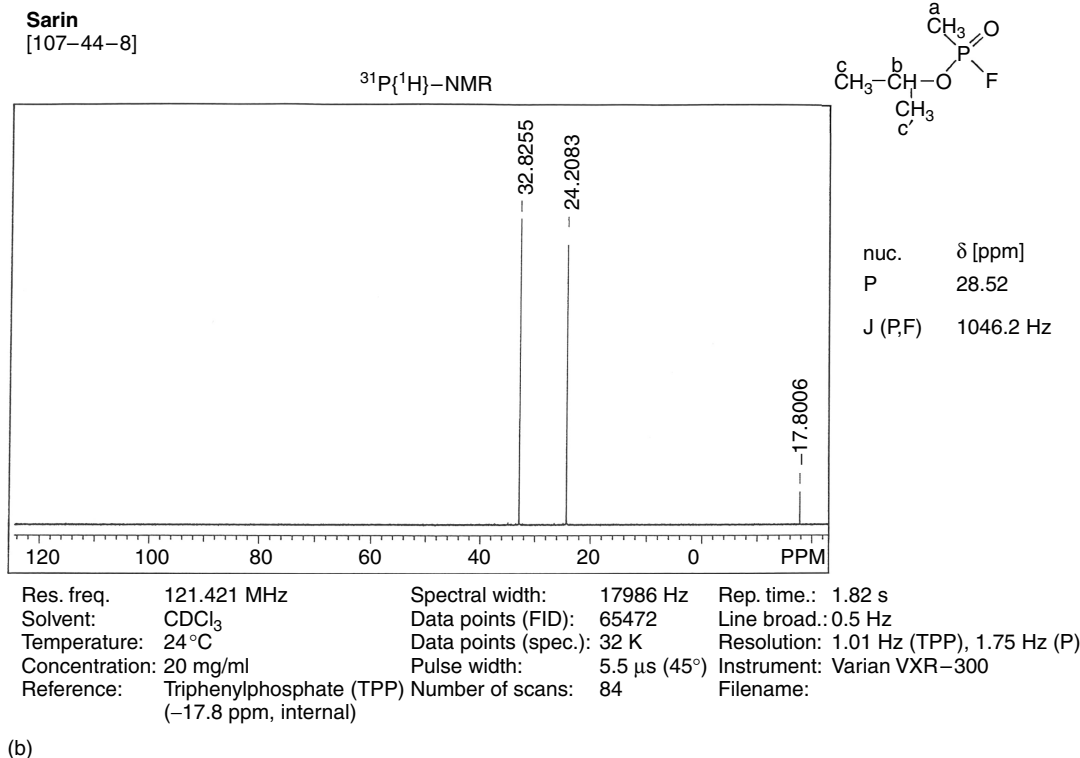


Figure 2. (b) $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of sarin (GB) in CDCl_3

from -80 to 5 ppm in ^{19}F NMR, and from -5 to 230 ppm in $^{13}\text{C}\{^1\text{H}\}$ NMR, all relative to the respective references TMS, H_3PO_4 , CFCl_3 , and TMS ($\delta = 0$ ppm). The number of points in the FID and in the real spectrum normally ranges from 32 to 128 k. The number of scans is selected so as to obtain a sufficient signal-to-noise ratio. Pulses of 45–90°; flip angles are used and the repetition time (pulse interval) is selected to be 3–6 s. Note that because the relaxation times of the nuclei of the target chemicals are normally not known, these conditions are a compromise, and should be modified according to the case: shorter repetition time if the nuclei relax fast (this can be approximately checked from the duration of the FID).

Experimental conditions for recording reference spectra from authentic chemicals are similar to those used for test samples. By way of example, Figure 2 shows the library NMR spectra of sarin (GB) from the Spiez Laboratory and Figure 3 shows those of tabun (GA) from the Finnish Institute for

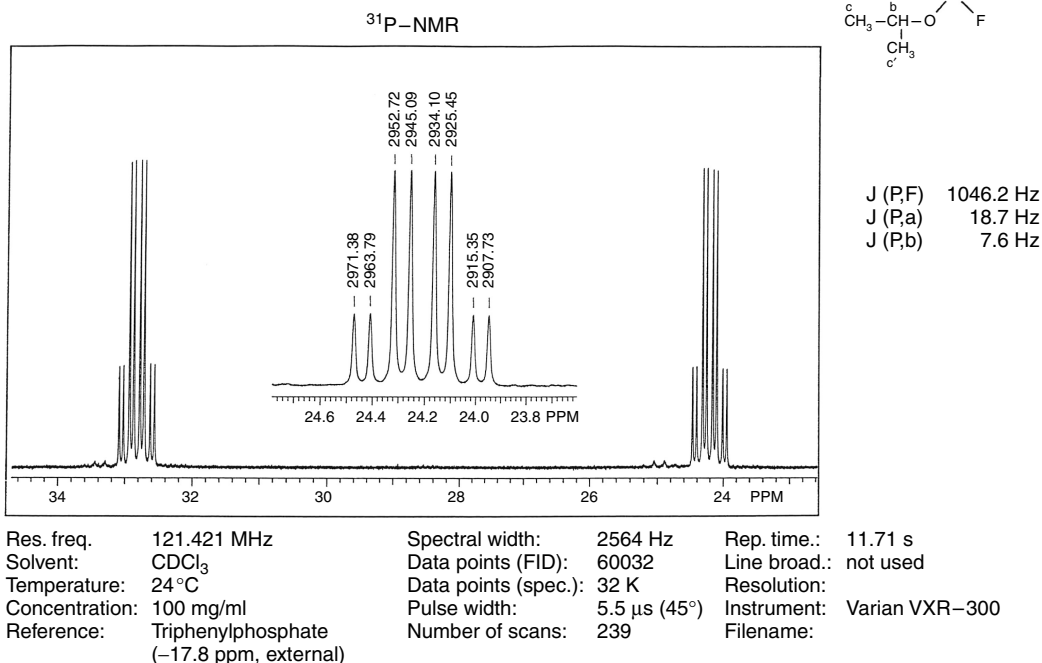
Verification of the Chemical Weapons Convention (VERIFIN). The spectra are presented in their original form (size reduced from A4) to show layout and format of library spectra that have been acceptable to the Organization for the Prohibition of Chemical Weapons (OPCW) Central Analytical Database (OCAD). The experimental conditions shown are the 'standard conditions' used by the laboratory for the particular experiment.

3.5.3 Processing of Data

Usually, the FID is Fourier transformed after the application of an exponential window function⁽²⁵⁾. The line-broadening factor is selected to provide a good S/N. As a rule, the line broadening is selected according to the width of a narrow singlet resonance – in ^1H NMR normally to a value from 0.1 to 0.5 Hz (cf. Figures 2 and 3). The resolution of the spectrum may be enhanced, though with reduced sensitivity, if the FID is multiplied, for example, by

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(c)

Figure 2. (c) ^{31}P NMR spectra of sarin (GB) in CDCl_3

a suitable Gaussian shape function, before Fourier transformation. The resolution enhancement is applied to better resolve the details of resonances, as in the case of resonance overlapping or in the case in which some coupling details need to be better resolved. Zero filling, that is, adding zero data points at the end of the FID prior to Fourier transformation, is sometimes necessary for proper representation of the line shape, particularly when resolution enhancement is performed. Baseline correction may be needed in cases in which very small intensity resonances need to be presented as large expansions.

3.5.4 Plotting of Data

A spectrum is plotted in its full width and as expanded regions. The spectrum is plotted on paper, always with a title that assigns a link between the actual spectrum, the sample (sample code), and the entry in the experiment logbook (e.g. a source reference code). The expanded regions of the

spectrum of the sample, the blank (if available), and the authentic reference chemical should be presented at the same scale and be sufficiently detailed. The experimental conditions are also printed out. Spectrum integration and peak picking are done where necessary.

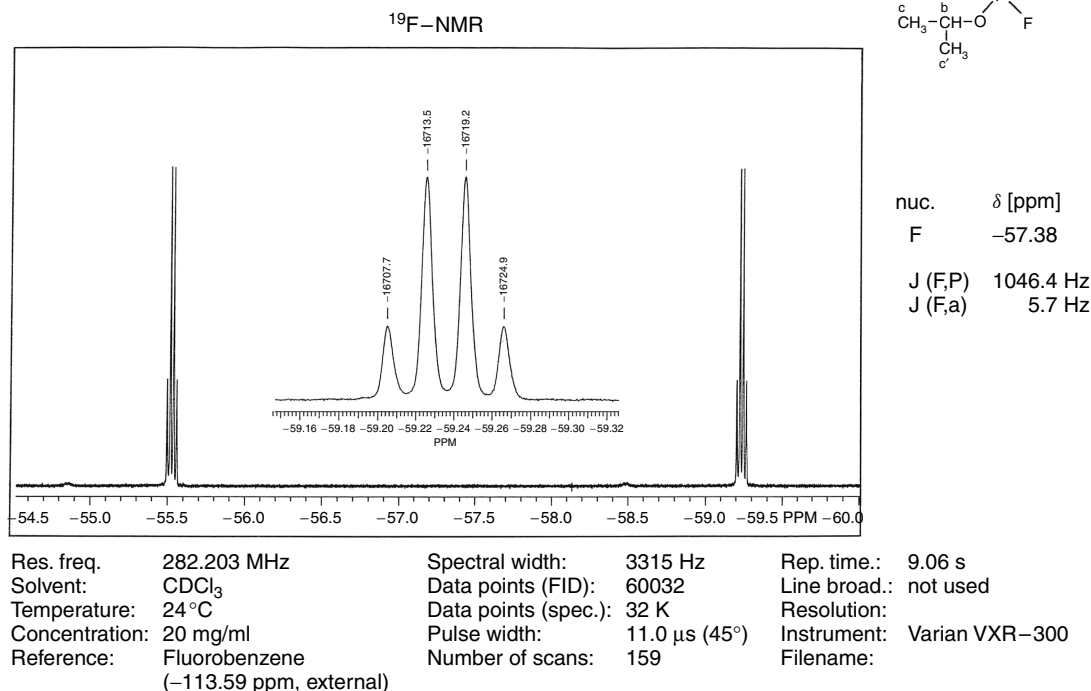
4 QUALITY CONTROL

4.1 Quality Management System

Work performed in a laboratory must be traceable. This is achieved when all relevant information from sample preparation to experiments and reporting of data are recorded on paper or stored in a computer. In practice, this requires a quality management system of the laboratory. Continued effort toward good quality practice in the laboratory and, if possible, national accreditation of the laboratory (testing methods) is recommended.

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[107–44–8]



(d)

Figure 2. (d) ^{19}F NMR spectra of sarin (GB) in CDCl_3

4.2 Data Quality

The quality of an NMR experiment can be assessed from the sample spectrum. In an optimal spectrum, a singlet resonance line is both narrow and symmetric, the S/N is high, and all resonances of interest are seen. The spectrum quality is a measure known to the laboratory after continuous work with the particular spectrometer (magnet) and probe head on different samples; thus, no limits can be suggested for the width of a singlet resonance. An experienced operator notices rather easily whether the problems with line shapes arise from a poor sample or from inadequate homogeneity adjustment.

Resonance overlap is a real problem in NMR when the technique is applied for chemical identification: details of all resonances of the identified chemical should be revealed (see Section 6.2). In particular, one should consider whether the outer

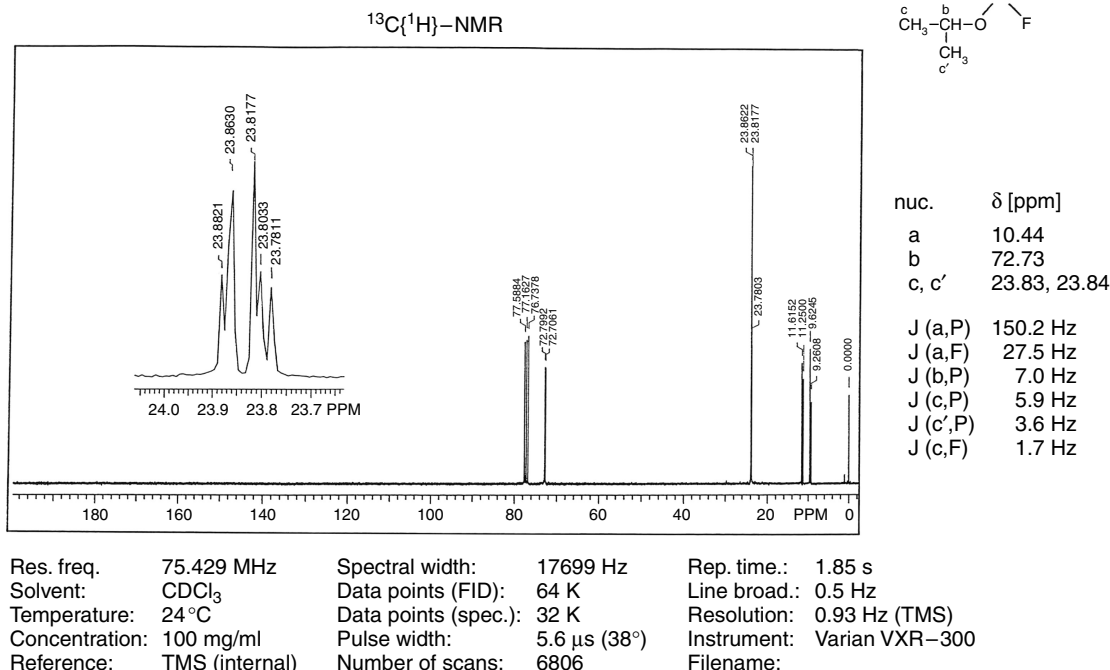
lines of multiplets are being seen, for example, those of the doublet of septets of the methine proton of GB (cf. Figure 2(a)); if the problem is poor S/N, more scans should be acquired in the experiment. Further homogeneity adjustment for a narrower line width and/or resolution enhancement may partially solve the problem of resonance overlap. General requirements for the competence of testing and calibration laboratories are set out in international standard ISO/IEC 17025.

4.3 Spectrometer Performance

In accordance with the quality control (QC) regime of the laboratory, the performance of the spectrometer should be checked and documented regularly. The specifications set by the spectrometer manufacturer are considered as the principal target to be obtained. Each experiment for the check of

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[107-44-8]



(e)

Figure 2. (e) $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of sarin (GB) in CDCl_3

spectrometer performance is logged in the experiment logbook. The spectrometer performance tests comprise checking of the ^1H line shape and sensitivity for a probe head and for any recorded X-nucleus. A ^1H resolution test may be unnecessary if the ^1H line shape test passes well. Other tests include checking of the radio-frequency field strengths of the observation and decoupling channels. A recommended practice for S/N determination in Fourier Transform (FT) NMR is described in standard 'Data Presentation Relating to High-Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy' (ASTM standards, E 386-90 (1999)). Spectrometer manufacturers provide instructions for S/N determination and the other tests (see also Braun *et al.* ⁽²³⁾).

4.4 Tests and Test Samples

Testing of the spectrometer performance requires a variety of test samples, usually provided by the

spectrometer manufacturer. A set of tests and test samples may be as follows:

- ^1H line shape, resolution, and spinning side band test: 3 % of chloroform in acetone- d_6 ;
- ^1H sensitivity: 0.1 % ethylbenzene in CDCl_3 ;
- ^{31}P sensitivity: 0.0485 M triphenylphosphate in CDCl_3 ;
- ^{19}F sensitivity: 0.05 % trifluorotoluene in CDCl_3 ;
- ^{13}C sensitivity: 10 % ethylbenzene in CDCl_3 ;
- ^1H resolution test: 5 % of *o*-dichlorobenzene in acetone- d_6 ;
- temperature calibration: 80 % of ethylene glycol in $\text{DMSO}-d_6$ and 4 % of methanol in methanol- d_4 .

Specifications for a probe head/field strength (magnet) combination are given by the spectrometer manufacturer for line shape, sensitivity, resolution, and radio-frequency field strengths. A specific test is considered 'passed' when the specification value is met.

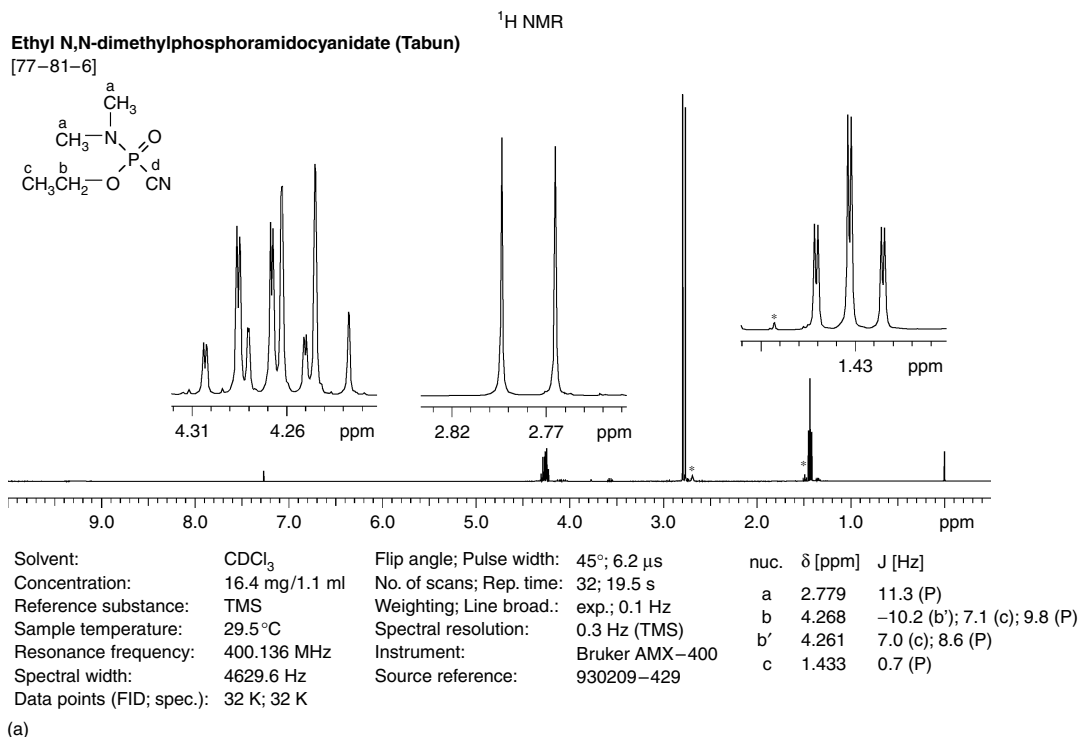


Figure 3. (a) ¹H, (b) ³¹P{¹H}, (c) ³¹P, and (d) ¹³C{¹H} NMR spectra of tabun (GA) in CDCl₃ from NMR Spectrum Library of VERIFIN

5 APPLICATIONS

5.1 Main One-dimensional (1-D) Experiments

The most important experiments (see Section 3.5) in the verification of CWC-related chemicals by NMR spectroscopy are ¹H, ³¹P{¹H}, ¹⁹F, ³¹P, and ¹³C{¹H} (listed in the order of importance in the inter-laboratory comparison/proficiency tests; Figure 1). These 1-D experiments are useful both for the identification of chemicals and in structure elucidation. The ¹H, ¹³C{¹H}, ¹⁹F, ³¹P{¹H}, and/or ³¹P NMR spectra can be recorded one after the other from the same NMR sample. The ¹H NMR experiment is most sensitive and often will be run first. Specific couplings in the ¹H spectrum may indicate the presence of phosphorus or fluorine, which is then confirmed by ³¹P{¹H} and/or ³¹P and ¹⁹F experiments. ¹³C{¹H} experiments are performed if the sample concentration is high

enough and if necessary. The literature can be consulted for general reading on NMR ^(3,4), ³¹P and multinuclear NMR ^(34,35), and practical performance of experiments ⁽²³⁾.

5.1.1 Screening

The presence of phosphorus- or fluorine-containing chemicals above the detection limit can be checked by running ³¹P{¹H} and ¹⁹F NMR experiments. Normally, in environmental samples, no organic phosphorus- or fluorine-containing chemicals exist in the background, which makes these experiments useful in screening. Fluorine observation has an advantage because the sensitivity is nearly as high as for protons. Although ¹H NMR also offers a sensitive screening method, there could be interference from a high proton background. The presence of phosphorus-containing chemicals could then not be ruled out simply by recording the ¹H spectrum.

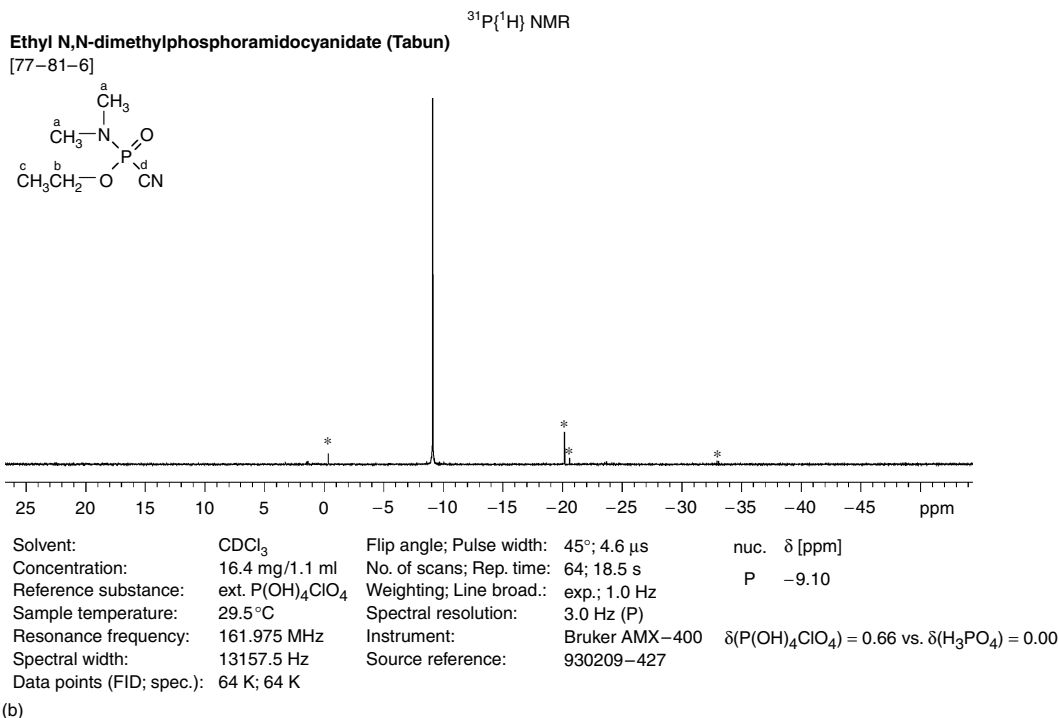


Figure 3. (b) $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of tabun (GA) in CDCl_3

5.1.2 ^1H Nuclear Magnetic Resonance

The ^1H NMR spectrum (Figures 2(a) and 3(a)) provides information on the number and type of protons (number of resonances, areas of resonances, δ_{H} (ppm)) in the molecule and, in the form of couplings [J (Hz)], may reveal other magnetic atoms (e.g. ^1H , ^{31}P , ^{19}F) one to four bonds away from a hydrogen atom. (The maximum distance of four bonds between the coupling nuclei should be considered approximate.) Note that the experimental conditions required for quantitative work, as given in Section 3.5.2, may not necessarily have been obtained, and the areas of the resonances should therefore be considered only approximate. In general, much longer repetition times are needed for quantitative conditions.

5.1.3 $^{31}\text{P}\{^1\text{H}\}$ and ^{31}P Nuclear Magnetic Resonance

The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum (Figures 2(b) and 3(b)) provides information on the number and type of

phosphorus atoms (number of resonances, areas of resonances, δ_{P}) in the molecule and, in the form of couplings, may reveal other magnetic atoms (e.g. ^{31}P , ^{19}F) one to four bonds from a phosphorus atom. (The maximum distance of four bonds between the coupling nuclei should be considered approximate.) The notation $^{31}\text{P}\{^1\text{H}\}$ means that, when ^{31}P is observed, protons are irradiated so that the J-coupling between protons and phosphorus disappears; however, the interaction with other magnetic nuclei remains.

The ^{31}P NMR spectrum (Figures 2(c) and 3(c)) provides information on the number and type of phosphorus atoms (number of resonances, areas of resonances, δ_{P}) in the molecule and, in the form of couplings, may reveal other magnetic atoms (e.g. ^1H , ^{31}P , ^{19}F) one to four bonds from a phosphorus atom. (The maximum distance of four bonds between the coupling nuclei should be considered approximate.)

Note that the $^{31}\text{P}\{^1\text{H}\}$ and ^{31}P NMR spectra are not quantitative, and the areas of the resonances should be considered only approximate. In addition,

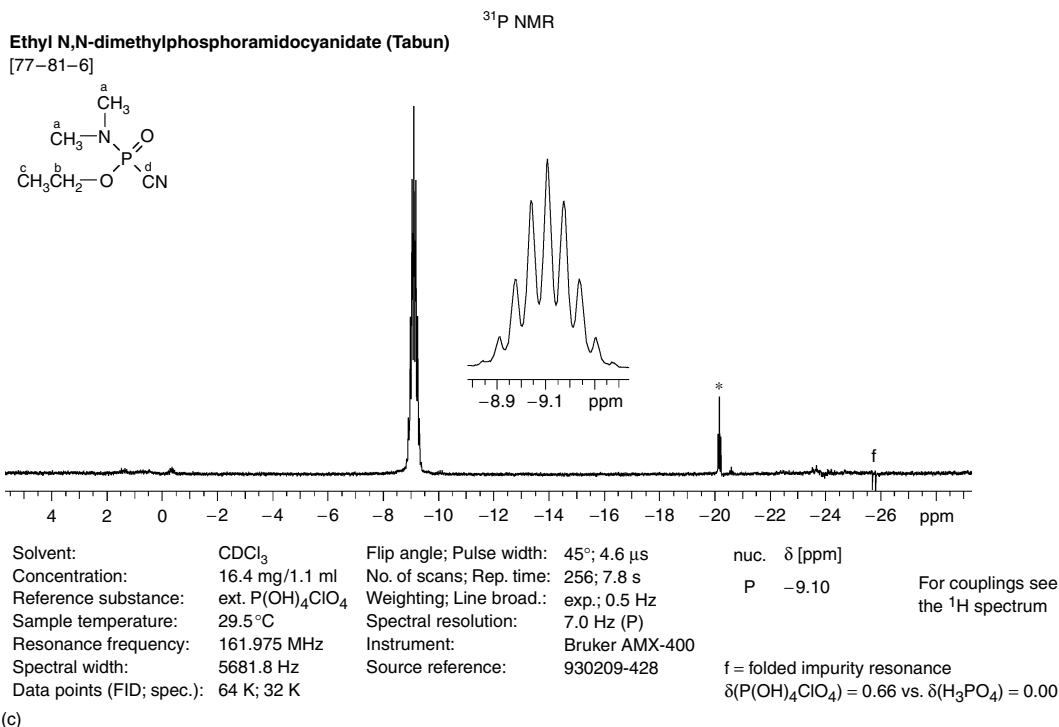


Figure 3. (c) ^{31}P NMR spectra of tabun (GA) in CDCl_3

a quantitative $^{31}\text{P}\{^1\text{H}\}$ experiment utilizes an inverse gated pulse sequence ^(36,37).

5.1.4 ^{19}F Nuclear Magnetic Resonance

The ^{19}F NMR spectrum (Figure 2(d)) provides information on the number and type of fluorine atoms (number of resonances, areas of resonances, δ_{F}) in the molecule and, in the form of couplings, may reveal other magnetic atoms (e.g. ^1H , ^{31}P , ^{19}F) one to four bonds from a fluorine atom. (The maximum distance of four bonds between the coupling nuclei should be considered approximate.) Note that the experimental conditions required for quantitative work, as given in Section 3.5.2, may not necessarily have been obtained, and the areas of the resonances should therefore be considered only approximate. In general, much longer repetition times are needed for quantitative conditions.

5.1.5 $^{13}\text{C}\{^1\text{H}\}$ Nuclear Magnetic Resonance

The $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (Figures 2(e) and 3(d)) provides information on the number and type of

carbons (number of resonances, areas of resonances, δ_{C}) in the molecule and, in the form of couplings, may reveal other magnetic atoms (e.g. ^{31}P , ^{19}F) one to four bonds from a carbon atom. (The maximum distance of four bonds between the coupling nuclei should be considered approximate.) Note that the $^{13}\text{C}\{^1\text{H}\}$ NMR spectra are not quantitative, and the areas of the resonances should be considered only approximate. In addition, a quantitative $^{31}\text{C}\{^1\text{H}\}$ experiment utilizes an inverse gated pulse sequence ⁽³⁶⁾.

5.2 $^1\text{H}\{^{31}\text{P}\}$, $^1\text{H}\{^{19}\text{F}\}$, $^1\text{H}\{^1\text{H}\}$, and $^1\text{H}-^{31}\text{P}$ Inverse Nuclear Magnetic Resonance experiments

In $^1\text{H}\{^{31}\text{P}\}$, $^1\text{H}\{^{19}\text{F}\}$, and $^1\text{H}\{^1\text{H}\}$ NMR experiments, a proton is observed, while phosphorus, fluorine, or proton is decoupled. The phosphorus and fluorine are decoupled either selectively or with broadband irradiation, while the proton is irradiated

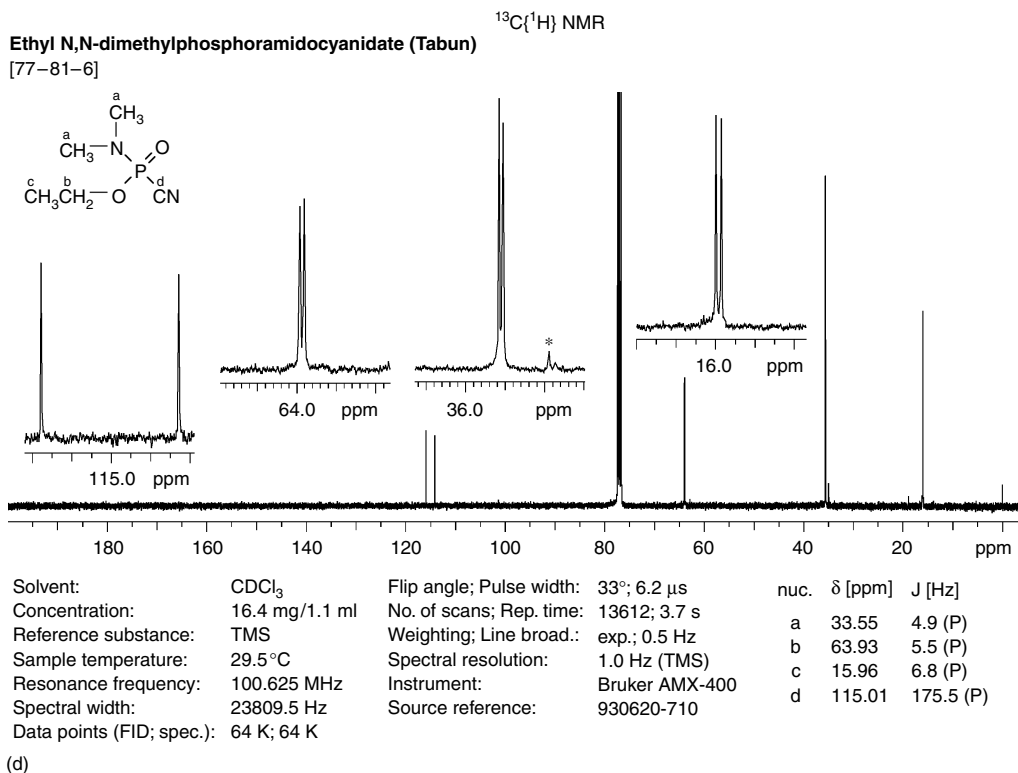


Figure 3. (d) $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of tabun (GA) in CDCl_3

selectively. In each case, a ^1H NMR spectrum simplified by the removal of the effect of the irradiated nucleus to the spectrum is obtained, that is, all nuclei that are coupled to the decoupled nucleus/nuclei exhibit a simplified resonance pattern. Comparison of the normal ^1H NMR spectrum with the decoupled spectrum may help in interpretation of the former and in elucidation of the molecular structure. Suitable 2-D experiments (homo- and heteronuclear correlation) provide comparable information; however, the 1-D experiments are quicker to perform and show the resonances in a more detailed manner.

Nonselective 1-D ^1H - ^{31}P inverse NMR spectroscopic experiments have been applied for analysis of CWC-related organophosphorus compounds⁽³⁸⁾. With these experiments, the alkyl substituent at the phosphorus can be determined with a better sensitivity than in the $^{31}\text{P}\{^1\text{H}\}$ experiment, and with elimination of strong background signals.

5.3 Relevant Two-dimensional Techniques

Two-dimensional (2-D) chemical shift correlation NMR techniques may be useful in supporting the verification analysis (Figure 1). In particular, they may be useful in the structure elucidation of an unknown CWC-related chemical. In 2-D spectra, the information is spread in the form of correlations over an xy -plane instead of being contained in a single spectrum curve as in 1-D spectra. Correlations connect resonances of the same or different types of nuclei of a molecule and give information on how close the nuclei yielding the resonances are. Most of the techniques make use of a J -coupling constant (a spin-spin coupling through chemical bonds). The rule that the size of the coupling constant (and the intensity of correlation) decreases as the distance between the coupled nuclei increases is roughly valid. Because of the two dimensions and the correlations, spectral analysis may be possible even in the presence of a strong background. The

Table 1. NMR spectral parameters of selected CWC-related chemicals

Trivial name; NATO code ^a ; IUPAC name [CAS] ^b (Schedule) ^c	Chemical shift δ [ppm] ^d (<i>multiplicity</i>) ^e , coupling constant J [Hz] ^f ; concentration and solvent, pH; chemical shift reference(s); remarks.
Sarin; GB; Isopropyl methylphosphonofluoridate [107-44-8] (1.A.1)	$\delta_{\text{Ha}} = 1.623(\text{dd})$, $\delta_{\text{Hb}} = 4.908(\text{dspd})$, $\delta_{\text{Hc}} = 1.375(\text{d})$, $\delta_{\text{Hd}} = 1.382(\text{d})$, $^2J_{\text{Ha,P}} = 18.7$, $^3J_{\text{Ha,F}} = 5.7$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 7.7$, $^4J_{\text{Hb,F}} = 0.4$, $\delta_{\text{Ca}} = 10.47(\text{dd})$, $\delta_{\text{Cb}} = 72.73(\text{d})$, $\delta_{\text{Cc}} = 23.840(\text{dd})$, $\delta_{\text{Cd}} = 23.845(\text{d})$, $^1J_{\text{Ca,P}} = 150.1$, $^2J_{\text{Ca,F}} = 27.5$, $^2J_{\text{Cb,P}} = 7.0$, $^3J_{\text{Cc,P}} = 5.9$, $^4J_{\text{Cd,F}} = 1.7$, $^3J_{\text{Cd,P}} = 3.5$, $\delta_{\text{F}} = -57.43(\text{dq})$, $^1J_{\text{F,P}} = 1046.3$, $\delta_{\text{P}} = 29.15(\text{ddq})$; 14.1 mg/1.1 mL CDCl_3 ; TMS, CFCl_3 , ext. H_3PO_4 .
Soman; GD; Pinacolyl methylphosphonofluoridate [96-64-0] (1.A.1)	<p>More abundant diastereomer: $\delta_{\text{Ha}} = 1.633(\text{dd})$, $\delta_{\text{Hb}} = 4.487(\text{ddq})$, $\delta_{\text{Hd}} = 0.936(\text{s})$, $\delta_{\text{He}} = 1.317(\text{d})$, $^2J_{\text{Ha,P}} = 18.7$, $^3J_{\text{Ha,F}} = 5.7$, $^3J_{\text{Hb,P}} = 7.9$, $^4J_{\text{Hb,F}} = 0.5$, $^3J_{\text{Hb,He}} = 6.4$, $\delta_{\text{Ca}} = 10.05(\text{dd})$, $\delta_{\text{Cb}} = 83.23(\text{d})$, $\delta_{\text{Cc}} = 34.89(\text{dd})$, $\delta_{\text{Cd}} = 25.43(\text{s})$, $\delta_{\text{Ce}} = 16.93(\text{s})$, $^1J_{\text{Ca,P}} = 151.3$, $^2J_{\text{Ca,F}} = 28.2$, $^2J_{\text{Cb,P}} = 8.1$, $^3J_{\text{Cc,P}} = 7.2$, $^4J_{\text{Ce,F}} = 1.5$, $^3J_{\text{Ce,P}} = 0.9$, $\delta_{\text{F}} = -55.48(\text{dq})$, $^1J_{\text{F,P}} = 1047.2$, $\delta_{\text{P}} = 29.75(\text{ddq})$.</p> <p>Less abundant diastereomer: $\delta_{\text{Ha}} = 1.636(\text{dd})$, $\delta_{\text{Hb}} = 4.438(\text{ddq})$, $\delta_{\text{Hd}} = 0.936(\text{s})$, $\delta_{\text{He}} = 1.328(\text{d})$, $^2J_{\text{Ha,P}} = 18.7$, $^3J_{\text{Ha,F}} = 5.7$, $^3J_{\text{Hb,P}} = 8.9$, $^3J_{\text{Hb,He}} = 6.4$, $\delta_{\text{Ca}} = 10.63(\text{dd})$, $\delta_{\text{Cb}} = 83.73(\text{d})$, $\delta_{\text{Cc}} = 34.80(\text{dd})$, $\delta_{\text{Cd}} = 25.43(\text{s})$, $\delta_{\text{Ce}} = 17.06(\text{s})$, $^1J_{\text{Ca,P}} = 150.7$, $^2J_{\text{Ca,F}} = 28.5$, $^2J_{\text{Cb,P}} = 7.9$, $^3J_{\text{Cc,P}} = 5.5$, $^3J_{\text{Ce,P}} = 2.0$, $^4J_{\text{Ce,F}} = 2.0$, $\delta_{\text{F}} = -58.33(\text{dq})$, $^1J_{\text{F,P}} = 1046.4$, $\delta_{\text{P}} = 28.73(\text{ddq})$; 75.5 mg/1.1 mL CDCl_3; TMS, CFCl_3, ext. H_3PO_4; molar ratio of diastereomers: approx. 55/45.</p>
Tabun; GA; <i>O</i> -Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate [77-81-6] (1.A.2)	$\delta_{\text{Ha}} = 2.779(\text{d})$, $\delta_{\text{Hb}} = 4.261(\text{m})$, $\delta_{\text{Hb}'} = 4.269(\text{m})$, $\delta_{\text{Hc}} = 1.433(\text{dt})$, $^3J_{\text{Ha,P}} = 11.3$, $^2J_{\text{Hb,Hb}'} = -10.2$, $^3J_{(\text{Hb,Hb}'),\text{Hc}} = 7.1$, $^3J_{\text{Hb,P}} = 8.6$, $^3J_{\text{Hb}',\text{P}} = 9.8$, $^4J_{\text{Hc,P}} = 0.7$, $\delta_{\text{Ca}} = 35.55$, $\delta_{\text{Cb}} = 63.93$, $\delta_{\text{Cc}} = 15.96$, $\delta_{\text{Cd}} = 115.01$, $^2J_{\text{Ca,P}} = 4.9$, $^2J_{\text{Cb,P}} = 5.5$, $^3J_{\text{Cc,P}} = 6.8$, $\delta_{\text{P}} = -9.02$; 16.4 mg/1.1 mL CDCl_3 ; TMS, ext. H_3PO_4 .
VX; <i>O</i> -Ethyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate [50782-69-9] (1.A.3)	$\delta_{\text{Ha}} = 1.783(\text{d})$, $\delta_{\text{Hb}} = 4.094(\text{ddq})$, $\delta_{\text{Hb}'} = 4.187(\text{ddq})$, $\delta_{\text{Hc}} = 1.347(\text{t})$, $\delta_{\text{Hd}} = 2.799(\text{m})$, $\delta_{\text{Hd}'} = 2.834(\text{m})$, $\delta_{\text{He}} = 2.684(\text{m})$, $\delta_{\text{He}'} = 2.703(\text{m})$, $\delta_{\text{Hf}} = 3.011(\text{sp})$, $\delta_{\text{Hg}} = 1.018(\text{d})$, $^2J_{\text{Ha,P}} = 15.6$, $^3J_{\text{Hb,P}} = 9.3$, $^2J_{\text{Hb,Hb}'} = -10.2$, $^3J_{(\text{Hb,Hb}'),\text{Hc}} = 7.1$, $^3J_{\text{Hb}',\text{P}} = 8.4$, $^3J_{\text{Hd,P}} = 12.4$, $^2J_{\text{Hd,Hd}'} = -12.5$, $^3J_{\text{Hd,He}} = 10.0$, $^3J_{\text{Hd,He}'} = 5.6$, $^3J_{\text{Hd}',\text{P}} = 12.6$, $^3J_{\text{Hd}',\text{He}} = 5.7$, $^3J_{\text{Hd}',\text{He}'} = 10.0$, $^2J_{\text{He,He}'} = -14.1$, $^3J_{\text{Hf,Hg}} = 6.6$, $\delta_{\text{Ca}} = 20.06(\text{d})$, $\delta_{\text{Cb}} = 61.15(\text{d})$, $\delta_{\text{Cc}} = 16.26(\text{d})$, $\delta_{\text{Cd}} = 31.62(\text{d})$, $\delta_{\text{Ce}} = 46.62(\text{d})$, $\delta_{\text{Cf}} = 48.92(\text{s})$, $\delta_{\text{Cg}} = 20.94(\text{s})$, $20.99(\text{s})$, $^1J_{\text{Ca,P}} = 110.4$, $^2J_{\text{Cb,P}} = 6.9$, $^3J_{\text{Cc,P}} = 7.1$, $^2J_{\text{Cd,P}} = 2.8$, $^4J_{\text{Ce,P}} = 4.6$, $\delta_{\text{P}} = 54.80(\text{m})$; 50 μl /0.8 mL CDCl_3 ; TMS, ext. H_3PO_4 ; 2 μl of tributylamine was added.
Mustard gas; HD; Bis(2-chloroethyl)sulfide [505-60-2] (1.A.4)	$\delta_{\text{Ha}} = 2.923(\text{XX}')$, $\delta_{\text{Hb}} = 3.645(\text{AA}')$, $^2J_{\text{Ha,Hb}} = -10.9$, $^2J_{\text{Hb,Hb}'} = -13.9$, $^3J_{\text{Ha,Hb}} = 9.5$, $^3J_{\text{Ha,Hb}'} = 5.9$, $\delta_{\text{Ca}} = 34.61$, $\delta_{\text{Cb}} = 43.04$; 17.0 mg/1.0 mL CDCl_3 ; TMS.

(continued overleaf)

Table 1. (continued)

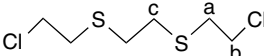
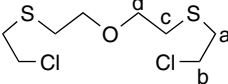
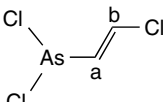
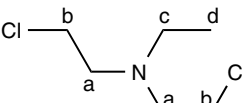

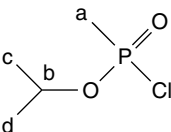
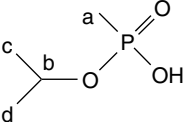
Trivial name; NATO code ^a ; IUPAC name [CAS] ^b (Schedule) ^c	Chemical shift δ [ppm] ^d (<i>multiplicity</i>) ^e , coupling constant J [Hz] ^f ; concentration and solvent, pH; chemical shift reference(s); remarks.
Sesquimustard; Q; Bis(2-chloroethylthio)ethane [3563-36-8] (1.A.4)	$\delta_{\text{Ha}} = 2.907(\text{XX}')$, $\delta_{\text{Hb}} = 3.645(\text{AA}')$, $^2J_{\text{Ha,Ha}'} = -10.9$, $^2J_{\text{Hb,Hb}'} = -13.8$, $^3J_{\text{Ha,Hb}} = 9.6$, $^3J_{\text{Ha,Hb}'} = 5.9$, $\delta_{\text{Ca}} = 34.50$, $\delta_{\text{Cb}} = 43.12$, $\delta_{\text{Cc}} = 32.65$; 21.3 mg/1.1 mL CDCl_3 ; TMS.
	
O-Mustard; T; Bis(2-chloroethylthio)ether [63918-89-8] (1.A.4)	$\delta_{\text{Ha}} = 2.924(\text{XX}')$, $\delta_{\text{Hb}} = 3.653(\text{AA}')$, $\delta_{\text{Hc}} = 2.762(t)$, $\delta_{\text{Hd}} = 3.653(t)$, $^2J_{\text{Ha,Ha}'} = -10.8$, $^2J_{\text{Hb,Hb}'} = -13.8$, $^3J_{\text{Ha,Hb}} = 9.8$, $^3J_{\text{Ha,Hb}'} = 5.8$, $^3J_{\text{Hc,Hd}} = 6.4$, $\delta_{\text{Ca}} = 34.82$, $\delta_{\text{Cb}} = 43.16$, $\delta_{\text{Cc}} = 32.04$, $\delta_{\text{Cd}} = 71.08$; 26.2 mg/1.0 mL CDCl_3 ; TMS.
	
Lewisite-1; L; 2-Chlorovinylchloroarsine [541-25-3] (1.A.5)	$\delta_{\text{Ha}} = 7.146(d)$, $\delta_{\text{Hb}} = 6.936(d)$, $^3J_{\text{Ha,Hb}} = 14.8$, $\delta_{\text{Ca}} = 139.27$, $\delta_{\text{Cb}} = 133.17$; 22.3 mg/1.1 mL CDCl_3 ; TMS.
	
Nitrogen mustard one; HN-1; Bis(2-chloroethyl)ethylamine [538-07-8] (1.A.6)	$\delta_{\text{Ha}} = 2.865(\text{XX}')$, $\delta_{\text{Hb}} = 3.507(\text{XX}')$, $\delta_{\text{Hc}} = 2.654(q)$, $\delta_{\text{Hd}} = 1.051(t)$, $^3J_{\text{Hc,Hd}} = 7.1$, $\delta_{\text{Ca}} = 55.97$, $\delta_{\text{Cb}} = 42.08$, $\delta_{\text{Cc}} = 48.52$, $\delta_{\text{Cd}} = 12.35$; 30 μl /0.8 mL CDCl_3 ; TMS.
	
DF; Methylphosphonyl difluoride [676-99-3] (1.B.9)	$\delta_{\text{Ha}} = 1.886(dt)$, $^2J_{\text{Ha,P}} = 19.4$, $^3J_{\text{Ha,F}} = 5.8$, $\delta_{\text{Ca}} = 8.49(dt)$, $^1J_{\text{Ca,P}} = 148.4$, $^2J_{\text{Cb,F}} = 22.5$, $\delta_{\text{F}} = -59.57(dq)$, $^1J_{\text{F,P}} = 1109.8$, $\delta_{\text{P}} = 24.47(tq)$; 20 μl /1.0 mL CDCl_3 ; TMS, CFCl_3 , ext. H_3PO_4 .
	
Chlorosarin; Isopropyl methylphosphonochloridate [1445-76-7] (1.B.11)	$\delta_{\text{Ha}} = 1.955(d)$, $\delta_{\text{Hb}} = 4.942(dsp)$, $\delta_{\text{Hc/Hd}} = 1.399(d)$, $\delta_{\text{Hd/Hc}} = 1.413(d)$, $^2J_{\text{Ha,P}} = 17.6$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 10.5$, $\delta_{\text{Ca}} = 20.71(d)$, $\delta_{\text{Cb}} = 73.35(d)$, $\delta_{\text{Cc/Cd}} = 23.39(d)$, $\delta_{\text{Cd/Cc}} = 24.01(d)$, $^1J_{\text{Ca,P}} = 130.8$, $^2J_{\text{Cb,P}} = 8.0$, $^3J_{\text{Cc/Cd,P}} = 5.0$, $^3J_{\text{Cd/Cc,P}} = 4.7$, $\delta_{\text{P}} = 39.21(dq)$; 50 μl /0.8 mL CDCl_3 ; TMS, ext. H_3PO_4 .
	
Isopropyl methylphosphonate [1832-54-8] (2.B.4)	$\delta_{\text{Ha}} = 1.480(d)$, $\delta_{\text{Hb}} = 4.671(dsp)$, $\delta_{\text{Hc}} = \delta_{\text{Hd}} = 1.334(d)$, $^2J_{\text{Ha,P}} = 17.9$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 8.3$, $\delta_{\text{Ca}} = 12.37(d)$, $\delta_{\text{Cb}} = 70.08(d)$, $\delta_{\text{Cc}} = \delta_{\text{Cd}} = 23.98(d)$, $^1J_{\text{Ca,P}} = 148.2$, $^2J_{\text{Cb,P}} = 6.5$, $^3J_{\text{(Cc,Cd),P}} = 4.4$, $\delta_{\text{P}} = 33.40(dq)$; 5 μl /0.8 mL CDCl_3 ; TMS, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.552(d)$, $\delta_{\text{Hb}} = 4.659(dsp)$, $\delta_{\text{Hc}} = \delta_{\text{Hd}} = 1.326(d)$, $^2J_{\text{Ha,P}} = 17.4$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 8.2$, $\delta_{\text{Ca}} = 13.79(d)$, $\delta_{\text{Cb}} = 74.41(d)$, $\delta_{\text{Cc}} = \delta_{\text{Cd}} = 25.83(d)$, $^1J_{\text{Ca,P}} = 139.0$, $^2J_{\text{Cb,P}} = 6.0$, $^3J_{\text{(Cc,Cd),P}} = 4.1$, $\delta_{\text{P}} = 32.21(dq)$; 10 μl /0.8 mL D_2O , pH 0; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.275(d)$, $\delta_{\text{Hb}} = 4.442(dsp)$, $\delta_{\text{Hc}} = \delta_{\text{Hd}} = 1.252(d)$, $^2J_{\text{Ha,P}} = 16.4$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 8.6$, $\delta_{\text{Ca}} = 14.97(d)$, $\delta_{\text{Cb}} = 71.25(d)$.
	

Table 1. (continued)

Trivial name; NATO code ^a ; IUPAC name [CAS] ^b (Schedule) ^c	Chemical shift δ [ppm] ^d (multiplicity) ^e , coupling constant J[Hz] ^f ; concentration and solvent, pH; chemical shift reference(s); remarks.
	$\delta_{\text{Cc}} = \delta_{\text{Cd}} = 26.15(d)$, $^1J_{\text{Ca,P}} = 137.2$, $^2J_{\text{Cb,P}} = 5.3$, $^3J_{(\text{Cc,Cd),P}} = 3.7$, $\delta_{\text{P}} = 26.22(dq)$; 10 $\mu\text{l}/0.8\text{ mL D}_2\text{O}$, pH 7.6; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.275(d)$, $\delta_{\text{Hb}} = 4.436(dsp)$, $\delta_{\text{Hc}} = \delta_{\text{Hd}} = 1.252(d)$, $^2J_{\text{Ha,P}} = 16.4$, $^3J_{\text{Hb,(Hc,Hd)}} = 6.2$, $^3J_{\text{Hb,P}} = 8.6$, $\delta_{\text{Ca}} = 14.96(d)$, $\delta_{\text{Cb}} = 71.24(d)$, $\delta_{\text{Cc}} = \delta_{\text{Cd}} = 26.13(d)$, $^1J_{\text{Ca,P}} = 136.9$, $^2J_{\text{Cb,P}} = 5.5$, $^3J_{(\text{Cc,Cd),P}} = 3.8$, $\delta_{\text{P}} = 26.22(dq)$; 10 $\mu\text{l}/0.8\text{ mL D}_2\text{O}$, pH 14; TSPSA, ext. H_3PO_4 .
Pinacolyl methylphosphonate [616-52-4] (2.B.4)	$\delta_{\text{Ha}} = 1.481(d)$, $\delta_{\text{Hb}} = 4.199(dq)$, $\delta_{\text{Hd}} = 0.919(s)$, $\delta_{\text{He}} = 1.286(d)$, $^2J_{\text{Ha,P}} = 17.9$, $^3J_{\text{Hb,P}} = 8.9$, $^3J_{\text{Hb,He}} = 6.4$, $^4J_{\text{He,P}} = 0.3$, $\delta_{\text{Ca}} = 12.36(d)$, $\delta_{\text{Cb}} = 80.89(d)$, $\delta_{\text{Cc}} = 34.88(d)$, $\delta_{\text{Cd}} = 25.58(s)$, $\delta_{\text{Ce}} = 16.88(s)$, $^1J_{\text{Ca,P}} = 149.6$, $^2J_{\text{Cb,P}} = 7.7$, $^3J_{\text{Cc,P}} = 6.3$, $^3J_{\text{Ce,P}} = 1.1$, $\delta_{\text{P}} = 33.52(dq)$; 5 $\mu\text{l}/0.8\text{ mL CDCl}_3$; TMS, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.562(d)$, $\delta_{\text{Hb}} = 4.188(dq)$, $\delta_{\text{Hd}} = 0.918(s)$, $\delta_{\text{He}} = 1.277(d)$, $^2J_{\text{Ha,P}} = 17.4$, $^3J_{\text{Hb,P}} = 8.6$, $^3J_{\text{Hb,He}} = 6.4$, $\delta_{\text{Ca}} = 13.87(d)$, $\delta_{\text{Cb}} = 85.01(d)$, $\delta_{\text{Cc}} = 36.93(d)$, $\delta_{\text{Cd}} = 27.46(s)$, $\delta_{\text{Ce}} = 19.03(d)$, $^1J_{\text{Ca,P}} = 149.6$, $^2J_{\text{Cb,P}} = 7.3$, $^3J_{\text{Cc,P}} = 6.1$, $\delta_{\text{P}} = 31.50(dq)$; 21 $\text{mg}/0.7\text{ mL D}_2\text{O}$, pH 0; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.275(d)$, $\delta_{\text{Hb}} = 3.959(dq)$, $\delta_{\text{Hd}} = 0.895(s)$, $\delta_{\text{He}} = 1.198(d)$, $^2J_{\text{Ha,P}} = 16.4$, $^3J_{\text{Hb,P}} = 9.1$, $^3J_{\text{Hb,He}} = 6.4$, $\delta_{\text{Ca}} = 15.28(d)$, $\delta_{\text{Cb}} = 82.14(d)$, $\delta_{\text{Cc}} = 36.95(d)$, $\delta_{\text{Cd}} = 27.81(s)$, $\delta_{\text{Ce}} = 19.20(d)$, $^1J_{\text{Ca,P}} = 138.0$, $^2J_{\text{Cb,P}} = 6.8$, $^3J_{\text{Cc,P}} = 5.3$, $\delta_{\text{P}} = 25.97(dq)$; 7.6 $\text{mg}/0.7\text{ mL D}_2\text{O}$, pH 7.6; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.275(d)$, $\delta_{\text{Hb}} = 3.954(dq)$, $\delta_{\text{Hd}} = 0.894(s)$, $\delta_{\text{He}} = 1.197(d)$, $^2J_{\text{Ha,P}} = 16.4$, $^3J_{\text{Hb,P}} = 9.1$, $^3J_{\text{Hb,He}} = 6.4$, $\delta_{\text{Ca}} = 15.27(d)$, $\delta_{\text{Cb}} = 82.11(d)$, $\delta_{\text{Cc}} = 36.93(d)$, $\delta_{\text{Cd}} = 27.80(s)$, $\delta_{\text{Ce}} = 19.20(d)$, $^1J_{\text{Ca,P}} = 137.8$, $^2J_{\text{Cb,P}} = 6.8$, $^3J_{\text{Cc,P}} = 5.5$, $\delta_{\text{P}} = 25.94(dq)$; 10.9 $\text{mg}/0.8\text{ mL D}_2\text{O}$, pH 14; TSPSA, ext. H_3PO_4 .
Methylphosphonic acid [993-13-5] (2.B.4)	$\delta_{\text{Ha}} = 1.543(d)$, $^2J_{\text{Ha,P}} = 17.5$, $\delta_{\text{Ca}} = 14.39(d)$, $^1J_{\text{Ca,P}} = 136.6$, $\delta_{\text{P}} = 31.47(q)$; 9.6 $\text{mg}/0.9\text{ mL D}_2\text{O}$, pH 0; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.206(d)$, $^2J_{\text{Ha,P}} = 16.1$, $\delta_{\text{Ca}} = 16.20(d)$, $^1J_{\text{Ca,P}} = 132.9$, $\delta_{\text{P}} = 24.49(q)$; 10.4 $\text{mg}/0.9\text{ mL D}_2\text{O}$, pH 7.6; TSPSA, ext. H_3PO_4 . $\delta_{\text{Ha}} = 1.075(d)$, $^2J_{\text{Ha,P}} = 15.5$, $\delta_{\text{Ca}} = 16.86(d)$, $^1J_{\text{Ca,P}} = 130.5$, $\delta_{\text{P}} = 20.93(q)$; 11.6 $\text{mg}/0.9\text{ mL D}_2\text{O}$, pH 14; TSPSA, ext. H_3PO_4 .
O-Ethyl methylthiophosphonate (2.B.4)	$\delta_{\text{Ha}} = 1.880(d)$, $\delta_{\text{Hb}'} = 4.201(ddq)$, $\delta_{\text{Hb}} = 4.142(ddq)$, $\delta_{\text{Hc}} = 1.348(dd)$, $^2J_{\text{Ha,P}} = 15.8$, $^2J_{\text{Hb,Hb}'} = -10.1$, $^2J_{\text{Hb,P}} = 9.0$, $^2J_{\text{Hb}',P}} = 10.7$, $^3J_{\text{Hb,Hc}} = 7.0$, $^3J_{\text{Hb}',Hc}} = 7.1$, $^4J_{\text{Hc,P}} = 0.2$, $\delta_{\text{Ca}} = 21.88(d)$, $\delta_{\text{Cb}} = 62.10(d)$, $\delta_{\text{Cc}} = 16.09(d)$, $^1J_{\text{Ca,P}} = 114.4$, $^2J_{\text{Cb,P}} = 6.8$, $^3J_{\text{Cc,P}} = 7.7$, $\delta_{\text{P}} = 89.95(m)$; 20 $\mu\text{l}/0.8\text{ mL CDCl}_3$; TMS, ext. H_3PO_4 .
N,N-Dimethylphosphoramidic dichloride [677-43-0] (2.B.5)	$\delta_{\text{Ha}} = 2.879(d)$, $^3J_{\text{Ha,P}} = 15.7$, $\delta_{\text{Ca}} = 37.02(d)$, $^2J_{\text{Ca,P}} = 3.6$, $\delta_{\text{P}} = 19.95(sp)$; 50 $\mu\text{l}/0.8\text{ mL CDCl}_3$; TMS, ext. H_3PO_4 .

(continued overleaf)

Table 1. (continued)

Trivial name; NATO code ^a ; IUPAC name [CAS] ^b (Schedule) ^c	Chemical shift δ [ppm] ^d (multiplicity) ^e , coupling constant J [Hz] ^f ; concentration and solvent, pH; chemical shift reference(s); remarks.
Benzilic acid; 2,2-Diphenyl-2-hydroxyacetic acid [76-93-7] (2.B.8)	$\delta_{\text{Hc}} = 7.468(m)$, $\delta_{\text{Hd}} = 7.337(m)$, $\delta_{\text{He}} = 7.319(m)$, $\delta_{\text{Hg,Hh}} = 5.08(bs)$, $^3J_{\text{Hc,Hd}} = 7.95$, $^4J_{\text{Hc,He}} = 1.24$, $^5J_{\text{Hc,Hd'}} = 0.58$, $^4J_{\text{Hc,He'}} = 2.12$, $^3J_{\text{Hd,He}} = 7.45$, $^4J_{\text{Hd,Hd'}} = 1.48$, $\delta_{\text{Ca}} = 81.02$, $\delta_{\text{Cb}} = 141.55$, $\delta_{\text{Cc}} = 127.41^s$, $\delta_{\text{Cd}} = 128.21^s$, $\delta_{\text{Ce}} = 128.25$, $\delta_{\text{Cf}} = 176.93$; 24.7 mg/1.0 mL CDCl_3 + c.a. 20 μl CD_3OD ; TMS.
<i>N,N</i> -Diisopropylaminoethane-2-ol [96-80-0] (2.B.11)	$\delta_{\text{Ha}} = 2.633(\text{XX}')$, $\delta_{\text{Hb}} = 3.460(\text{AA}')$, $\delta_{\text{Hc}} = 3.044(\text{sp})$, $\delta_{\text{Hd}} = 1.029(d)$, $\delta_{\text{OH}} = 3.14(b)$, $^3J_{\text{Hc,Hd}} = 6.6$, $\delta_{\text{Ca}} = 45.26$, $\delta_{\text{Cb}} = 58.36$, $\delta_{\text{Cc}} = 47.61$, $\delta_{\text{Cd}} = 20.92$; 40 μl /0.8 mL CDCl_3 ; TMS. $\delta_{\text{Ha}} = 3.288(\text{XX}')$, $\delta_{\text{Hb}} = 3.886(\text{AA}')$, $\delta_{\text{Hc}} = 3.776(\text{sp})$, $\delta_{\text{Hd}} = 1.351$, $1.364(d)$, $^3J_{\text{Hc,Hd}} = 6.6$, $\delta_{\text{Ca}} = 51.40$, $\delta_{\text{Cb}} = 60.22$, $\delta_{\text{Cc}} = 58.54$, $\delta_{\text{Cd}} = 19.28$, 20.99; 20 μl /0.8 mL D_2O , pH 0.5; TSP- d_4 . $\delta_{\text{Ha}} = 3.297(\text{XX}')$, $\delta_{\text{Hb}} = 3.899(\text{AA}')$, $\delta_{\text{Hc}} = 3.785(\text{sp})$, $\delta_{\text{Hd}} = 1.371(d)$, $^3J_{\text{Hc,Hd}} = 6.6$, $\delta_{\text{Ca}} = 51.28$, $\delta_{\text{Cb}} = 60.15$, $\delta_{\text{Cc}} = 58.37$, $\delta_{\text{Cd}} = 20.02$; 23 μl /0.8 mL D_2O , pH 8.4; TSP- d_4 . $\delta_{\text{Ha}} = 2.590(\text{XX}')$, $\delta_{\text{Hb}} = 3.564(\text{AA}')$, $\delta_{\text{Hc}} = 3.020(\text{sp})$, $\delta_{\text{Hd}} = 1.036(d)$, $^3J_{\text{Hc,Hd}} = 6.5$, $\delta_{\text{Ca}} = 50.13$, $\delta_{\text{Cb}} = 64.78$, $\delta_{\text{Cc}} = 52.71$, $\delta_{\text{Cd}} = 21.66$; 20 μl /0.8 mL D_2O , pH 12.8; TSP- d_4 .
Thiodiglycol; Bis(2-hydroxyethyl)sulfide [111-48-8] (2.B.13)	$\delta_{\text{Ha}} = 2.757(t)$, $\delta_{\text{Hb}} = 3.755(t)$, $^3J_{\text{Ha,Hb}} = 6.3$, $\delta_{\text{Ca}} = 36.10$, $\delta_{\text{Cb}} = 63.09$; 9.7 mg/0.6 mL D_2O , pH 6.5; TSPSA.
Diethyl phosphite [762-04-9] (3.B.11)	$\delta_{\text{Ha}} = 6.811(d)$, $\delta_{\text{Hb'}} = 4.157(ddq)$, $\delta_{\text{Hb}} = 4.148(ddq)$, $\delta_{\text{Hc}} = 1.368(t)$, $^1J_{\text{Ha,P}} = 692.4$, $^2J_{\text{Hb,Hb'}} = -10.2$, $^2J_{\text{Hb,P}} = 8.9$, $^2J_{\text{Hb',P}} = 9.3$, $^3J_{\text{Hb,Hc}} = 7.1$, $^3J_{\text{Hb',Hc}} = 7.1$, $\delta_{\text{Cb}} = 61.82(d)$, $\delta_{\text{Cc}} = 16.34(d)$, $^2J_{\text{Cb,P}} = 5.6$, $^3J_{\text{Cc,P}} = 6.2$, $\delta_{\text{P}} = 7.88(dqu)$; 20 μl /0.8 mL CDCl_3 ; TMS, ext. H_3PO_4 .
Ethylthiodiethanolamine [139-87-7] (3.B.15)	$\delta_{\text{Ha}} = 2.646(\text{XX}')$, $\delta_{\text{Hb}} = 3.613(\text{AA}')$, $\delta_{\text{Hc}} = 2.633(q)$, $\delta_{\text{Hd}} = 1.046(t)$, $\delta_{\text{OH}} = 3.29(b)$, $^3J_{\text{Hc,Hd}} = 7.1$, $\delta_{\text{Ca}} = 55.53$, $\delta_{\text{Cb}} = 59.66$, $\delta_{\text{Cc}} = 48.22$, $\delta_{\text{Cd}} = 11.62$; 40 μl /0.8 mL CDCl_3 ; TMS. $\delta_{\text{Ha}} = 3.38(\text{XX}')$, $\delta_{\text{Hb}} = 3.934(\text{AA}')$, $\delta_{\text{Hc}} = 3.370(q)$, $\delta_{\text{Hd}} = 1.327(t)$, $^3J_{\text{Hc,Hd}} = 7.3$, $\delta_{\text{Ca}} = 57.04$, $\delta_{\text{Cb}} = 58.20$, $\delta_{\text{Cc}} = 51.95$, $\delta_{\text{Cd}} = 10.89$; 40 μl /0.8 mL D_2O , pH 0.5; TSP- d_4 .

analysis of mixtures is more straightforward with 2-D techniques because the correlations do not overlap as readily as do the resonances in a 1-D spectrum.

By way of example, useful 2-D techniques are homonuclear correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC) spectroscopy, and heteronuclear multiple quantum coherence/total

correlation spectroscopy (HMQC/TOCSY) (1,2,23). An excellent technique is a proton-detected proton-phosphorus correlation experiment with combined heteronuclear single quantum coherence (HSQC) and TOCSY that has been used for the analysis of CWC-related chemicals during an international interlaboratory proficiency test (13). In this technique, protons of an alkylphosphonofluoridate,

Table 1. (continued)

Trivial name; NATO code ^a ; IUPAC name [CAS] ^b (Schedule) ^c	Chemical shift δ [ppm] ^d (multiplicity) ^e , coupling constant J [Hz] ^f ; concentration and solvent, pH; chemical shift reference(s); remarks.
Structure	
	$\delta_{\text{Ha}} = 3.378(\text{XX}')$, $\delta_{\text{Hb}} = 3.942(\text{AA}')$, $\delta_{\text{Hc}} = 3.362(q)$, $\delta_{\text{Hd}} = 1.334(t)$, $^3J_{\text{Hc,Hd}} = 7.3$, $\delta_{\text{Ca}} = 56.94$, $\delta_{\text{Cb}} = 58.21$, $\delta_{\text{Cc}} = 51.81$, $\delta_{\text{Cd}} = 10.84$; 40 μL /0.8 mL D ₂ O, pH 7.7; TSP-d ₄ . $\delta_{\text{Ha}} = 2.678(\text{XX}')$, $\delta_{\text{Hb}} = 3.685(\text{AA}')$, $\delta_{\text{Hc}} = 2.617(q)$, $\delta_{\text{Hd}} = 1.039(t)$, $^3J_{\text{Hc,Hd}} = 7.2$, $\delta_{\text{Ca}} = 57.51$, $\delta_{\text{Cb}} = 61.54$, $\delta_{\text{Cc}} = 50.88$, $\delta_{\text{Cd}} = 13.05$; 40 μL /0.8 mL D ₂ O, pH 12.5; TSP-d ₄ .

^a Standard NATO agreement (STANAG) code.^b Chemical Abstracts Service registry number.^c Schedule number, see Table 1.^d Chemical shifts are given relative to $\delta_{\text{H}}(\text{TMS}) = \delta_{\text{H}}(\text{TSP-d}_4) = 0.00$, $\delta_{\text{H}}(\text{TSPSA}) = 0.015$, $\delta_{\text{C}}(\text{TMS}) = \delta_{\text{C}}(\text{TSP-d}_4) = \delta_{\text{C}}(\text{TSPSA}) = 0.00$, $\delta_{\text{F}}(\text{CFCl}_3) = 0.00$, and $\delta_{\text{P}}(\text{H}_3\text{PO}_4) = 0.00$ ppm.^e Multiplicity: b = broad, d = doublet, dd = doublet of doublets, ddq = doublet of doublet of quartets, dq = doublet of quartets, dqu = doublet of quintets, dspd = doublet of septet of doublets, dt = doublet of triplets, m = multiplet, q = quartet, s = singlet, sp = septet, t = triplet, AA' = AA' part of an AA'XX' spin system, XX' = XX' part of an AA'XX' spin system. If no multiplicity is given, the resonance appears as singlet.^f Only the couplings $^2J_{\text{HH}}$ are marked negative; all others are marked positive even though they may be negative.^g Tentative assignment.

for example, show correlations to the phosphorus nucleus of the molecule, which is then used to assign from the 1-D ^1H spectrum the resonances that belong to this particular chemical. A 2-D NMR spectrum might also serve as a library or reference spectrum in the identification of a chemical.

5.4 Scheduled Chemicals

Suitability of NMR spectroscopy and the detection limits for the analysis of the Schedule 1–3 chemicals ⁽¹⁶⁾ are summarized below. Riot-control agents and vomiting agents are normally soluble in organic solvents, and are readily analyzed by NMR ^(11,39).

5.4.1 Schedule 1 Chemicals

Almost all Schedule 1 chemicals are soluble in the organic NMR solvents used in verification (see Section 3.1) and can be analyzed by NMR spectroscopy. Saxitoxin (1.A.7) and ricin (1.A.8) differ from the others. Both are derived from natural sources – the former is a paralytic shellfish poison and the latter, a glycoprotein toxin ⁽⁴⁰⁾. Analytical methods (ROPs) have not been established for either chemical. NMR data

of saxitoxin in D₂O has been reported ^(41,42). By way of example, Table 1 presents the NMR spectral parameters of sarin (GB), soman (GD), tabun (GA), *O*-Ethyl *S*-2-diisopropylaminoethyl methylphosphonothiolate (VX), mustard gas (HD), sesquimus-tard (Q), *O*-mustard (T), lewisite-1 (L), nitrogen mustard one (HN-1), methylphosphonyldifluoride, and chlorosarin.

5.4.2 Schedule 2 Chemicals

All Schedule 2 chemicals are analyzable by NMR, except arsenic trichloride, which does not possess a useful nucleus. Either an organic solvent or D₂O is used. Note that the hydrolysis products of alkylphosphonofluoridates (1.A.1), alkylphosphonothiolates (1.A.3), alkylphosphonyl difluorides (1.B.9), alkylphosphonites (1.B.10), chlorosarin (1.B.11), and chlorosoman (1.B.12), which have an alkyl group (Me, Et, *n*-Pr, or *i*-Pr) linked directly to a phosphorus, all belong to Schedule 2, group B.4, and NMR analysis for them is made either in D₂O or in an organic solvent. By way of example, NMR spectral parameters of isopropyl methylphosphonate, pinacolyl methylphosphonate, methylphosphonic acid, *O*-ethyl methylthiophosphonate, *N,N*-dimethylphosphoramidic dichloride,

benzilic acid, *N,N*-Diisopropylaminoethane-2-ol, and thiodiglycol are presented in Table 1.

5.4.3 Schedule 3 Chemicals

Most of the Schedule 3 chemicals are readily analyzed by NMR: trimethyl phosphite, triethyl phosphite, dimethyl phosphite, diethyl phosphite, ethyldiethanolamine, methyldiethanolamine, and triethanolamine. Organic solvents or D₂O are used. Although chloropicrine and the gases phosgene (3.A.1), cyanogen chloride (3.A.2), and hydrogen cyanide (3.A.3) can also be analyzed by NMR, their identification by other methods is more reliable. Phosphorus oxychloride (3.B.5), phosphorus trichloride (3.B.6), and phosphorus pentachloride (3.B.7) react readily with moisture, but, in principle, can be analyzed by NMR in a dry organic solvent. Sulfur monochloride (3.B.12), sulfur dichloride (3.B.13), and thionylchloride (3.B.14) do not possess an NMR nucleus suitable for verifying them. By way of example, NMR spectral parameters of diethylphosphite and ethyldiethanolamine are presented in Table 1.

5.5 Detection Limits

The detection limit of a chemical in NMR depends on its NMR properties (e.g. number of equivalent nuclei, presence and extent of couplings), the magnetic field strength of the spectrometer, the number of pulses, the flip angle used, the observed nucleus and its relaxation, the NMR tube diameter, the sample matrix, and the magnet shimming. Detection limits in ¹H, ¹³C, ¹⁹F, and ³¹P observation should all be determined, remembering, however, that the detection limit for a reference chemical does not guarantee the same detection limit for the chemical in a real sample. Typically, the detection limit of a chemical when ¹H is observed at 400 MHz or ¹⁹F at 376 MHz may be 1 µg/mL (1 ppm). Almost one order more of the analyte is required for phosphorus observation (5 ppm at 162 MHz), and recording of ¹³C{¹H} spectra may be futile in concentrations below 100 µg/mL (100 ppm).

6 ANALYSIS

6.1 Acquired NMR Data

The spectral data required to support an identification by NMR are the sample spectrum, the reference spectrum, and the spectrum of a blank sample (if available). All these should be recorded under comparable conditions. The reference spectrum may be from a library or database, it may be recorded from the authentic reference chemical, or it may be a spectrum of a closely related chemical; in this last case, spectral interpretation must be enclosed ⁽⁴³⁾. Other 1-D and 2-D NMR spectra (see Sections 5.2 and 5.3) may have been recorded from the test sample to support the interpretation and identification.

6.2 Identification Criteria

As the main rule in verification, unambiguous identification of a CWC-related chemical should be based on at least two analytical, preferably different spectrometric techniques (e.g. gas chromatography/mass spectrometry (GC/MS) and NMR) giving consistent results ⁽⁴³⁾. The NMR spectral parameters for a chemical should agree within certain limits with those of a database or spectrum library or with the parameters derived from the spectrum of an authentic reference chemical. According to the identification criteria ⁽¹⁹⁾, the values of chemical shifts δ_H , δ_C , and δ_F should agree with the corresponding values in the database or library or the values of an authentic reference chemical within limits ± 0.1 ppm, and those of δ_P within ± 1 ppm. The values of coupling constants $^nJ_{H,H}$, $^nJ_{H,F}$, $^nJ_{H,P}$, $^nJ_{C,P}$, $^nJ_{C,F}$, and $^nJ_{P,F}$ should agree within limits of ± 0.5 Hz. In addition, the resonance patterns and their intensities must match. Normally, when the conditions of the test sample and the reference sample are comparable, the differences in the NMR parameters are much less than suggested above; sometimes, within reasonable limits and when credibly explained, greater differences may be acceptable.

Sample conditions (solvent, pH in water, chemical shift references, concentration) and experimental conditions should correspond in the sample and reference sample. Sample concentration and the

experimental conditions are less critical for the NMR spectral parameters.

Other chemicals (matrix) present in the sample, especially at low analyte concentration, may affect the NMR spectral parameters and how the resonances are revealed. A general requirement for a spectrum acceptable for identification is that resonances of other chemicals do not overlap with resonances of the identified chemical. Partial overlapping may be acceptable if the resonance of the identified chemical can still be credibly explained. Where insufficient data are obtained, for example owing to severe overlapping, the resonances revealed may still be useful in supporting identifications based on other analytical techniques.

6.3 Interpretation

The sample spectra are compared with the corresponding spectra recorded from the blank sample (if available), and the differences are investigated. If the blank sample represents the background well, the resonances that are not from the background can be determined directly. Obtaining a representative blank sample from a contaminated environmental site will not always be a realistic possibility, however. Initial interpretation of the spectra may reveal a need for further sample preparation and experiments (Figure 1).

Indicative structural details may be revealed; for example, methyl, ethyl, propyl, and isopropyl groups directly linked to phosphorus will show different resonance patterns in ^1H NMR spectra. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum can reveal the presence of phosphorus-containing chemicals, and in the case of a large coupling constant (due to a P–F bond; see Figure 2(b)), also the presence of fluorine. The number of resonances and their integrated areas may give an idea of the number of chemicals present.

Note that nerve agent homologues that possess the same atoms one to three bonds apart from phosphorus may show similar phosphorus or fluorine resonances at comparable chemical shifts. Thus, an analysis based solely on ^{31}P and/or ^{19}F NMR spectra may lead to an erroneous identification; it should not be based solely on $^{31}\text{P}\{^1\text{H}\}$ NMR spectra either. The situation can become different when the sample is spiked with the authentic chemical, and then

identification may be obtained. However, in any identification by NMR spectroscopy, ^1H NMR is recommended as the first method of choice.

In the case of an unknown chemical, or where resonance overlap occurs, it may be necessary to call upon the full arsenal of NMR methods. To confirm a heteronuclear coupling, the normal ^1H NMR spectrum is compared with $^1\text{H}\{^{19}\text{F}\}$ and/or $^1\text{H}\{^{31}\text{P}\}$ NMR spectra. After this, and, in particular, where a strong background is present, the various 2-D NMR spectra are recorded. Homonuclear chemical shift correlation experiments such as COSY and TOCSY (or some of their variants) provide information on coupled protons, even networks of protons ⁽¹⁾, while the inverse detected heteronuclear correlation experiments such as HMQC and HMQC/TOCSY provide similar information but only for protons coupling to heteronuclei, for example, the pairs ^1H – ^{31}P and ^1H – ^{13}C . Although interpretation of these data provides abundant information on the molecular structure, the results obtained with other analytical or spectrometric techniques must be taken into account as well. The various methods of MS and gas chromatography/Fourier transform infrared (GC/FTIR) spectroscopy supply complementary information to fully resolve or confirm the structure. Unambiguous identification of an unknown chemical requires consistent results from all spectrometric techniques employed.

6.4 Data Evaluation

In the evaluation of acquired NMR data, one must consider whether the quality of the spectra and other accompanying information are sufficient for identification, or whether the data should only be regarded as support to an identification made by other techniques (Figure 1). One must consider whether all resonances of the chemical were revealed (S/N; occurrence and significance of overlapping resonances of other chemicals), whether the resonance patterns in the sample and reference spectra match, and whether they are correctly interpreted, whether the sample conditions were comparable to those of the reference sample, whether the reference data are of adequate quality, and in the case of interpretation, how the related data supported the identification ⁽⁴³⁾.

6.5 Identification

Identification by NMR of a CWC-related chemical is made through reference to spectra (^1H , $^{31}\text{P}\{^1\text{H}\}$, ^{31}P , ^{19}F and/or $^{13}\text{C}\{^1\text{H}\}$) included in a spectral library, through comparison with a spectrum of the authentic reference chemical, or through spiking the test sample with an authentic reference chemical. The spectrum of a blank sample (if available) is examined for comparison. Identification of a chemical is obtained if all the identification criteria are met. Identification made by spiking the sample with the suspected chemical requires exact overlapping of the resonances. By way of example, the ^1H NMR spectra for the identification of thiodiglycol in a soil sample in the Fourth Official OPCW Proficiency Test are presented in Figure 4.

6.6 NMR Spectra of Sarin and Tabun

The ^1H , $^{31}\text{P}\{^1\text{H}\}$, ^{31}P , $^{13}\text{C}\{^1\text{H}\}$, and/or ^{19}F NMR spectra of sarin and tabun are shown in Figures 2 and 3. The spectra of sarin (Figure 2(a)–(e)) are from the Atlas of the NMR Spectra of the Spiez Laboratory and the spectra of tabun (Figure 3(a)–(d)) from VERIFIN's NMR Spectrum Library. Interpretations of the spectra are given below. The chemical shifts of the nuclei are not

discussed since they can be explained with the use of the common chemical shift correlation rules found in textbooks^(3,4,34).

In the ^1H NMR spectrum of sarin (Figure 2(a)), protons H-a yield a doublet of doublets owing to couplings to phosphorus and fluorine. This is typical of all *O*-alkyl methylphosphonofluoridates. Proton H-b shows a doublet of septets due to coupling to phosphorus and the six methyl protons. Protons H-c and H-c' are diastereotopic and resonate as separate doublets; the intensity of the former is lower because of the different long-range couplings to phosphorus and fluorine. The $^{31}\text{P}\{^1\text{H}\}$ spectrum (Figure 2(b)) shows a typical doublet due to a large $^1J_{\text{P,F}}$. The ^{31}P spectrum (Figure 2(c)) shows, in addition, doublets of quartets through couplings of phosphorus to H-b and H-a, respectively. In general, this kind of resonance is interpreted as indicating the structure $\text{CH}-\text{O}-\text{P}(=\text{O})(\text{CH}_3)\text{F}$, and does not necessarily identify sarin. The ^{19}F spectrum (Figure 2(d)) reveals a doublet of quartets owing to couplings with the fluorine and protons H-a, thus giving an indication of substructure $-\text{O}-\text{P}(=\text{O})(\text{CH}_3)\text{F}$. Carbons C-a and C-c (Figure 2(e)) yield a doublet of doublets owing to couplings to phosphorus and fluorine, and C-b and C-c' doublets owing to coupling to phosphorus.

In the ^1H NMR spectrum of tabun (Figure 3(a)), protons H-a yield a typical doublet owing to

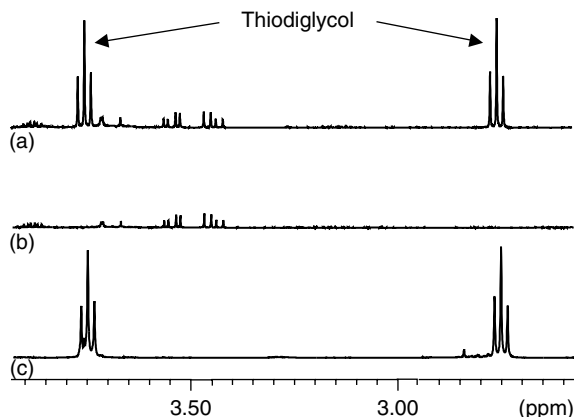


Figure 4. Expanded regions of ^1H NMR spectra recorded from extracts of (a) a soil sample (pH 8.2), (b) a blank soil sample in the Fourth Official OPCW Proficiency Test (May 1998). The soils were extracted with water, the extract was concentrated, and the remaining water was replaced with D_2O , and (c) spectrum of authentic thiodiglycol (pH 6.3) in D_2O , which was identified in the soil sample. The ^1H chemical shifts were 2.763 and 3.758 in (a) and 2.757 and 3.755 in (c); the coupling constant was 6.3 Hz in both spectra. The experimental conditions in all spectra were similar

coupling with phosphorus. Protons H-b show a resonance that resembles a doublet of quartets; however, the protons are diastereotopic with a small chemical shift difference and reveal a higher-order resonance. The couplings and chemical shifts can be analyzed with the use of a spectral analysis program. Protons H-c yield a doublet of triplets with a four-bond coupling to phosphorus and almost the same magnitude couplings to H-b and H-b'. The $^{31}\text{P}\{^1\text{H}\}$ spectrum (Figure 3(b)) shows a singlet at a chemical shift typical for alkyl *N,N*-dialkylphosphoramidocyanidates. The ^{31}P resonance (Figure 3(c)) resembles a nonet; however, the correct interpretation is a doublet (8.6 Hz) of doublets (9.8 Hz) of septets (11.3 Hz), but because of the broad lines, these details are hidden and only the average splitting is seen. All resonances in the $^{13}\text{C}\{^1\text{H}\}$ spectrum (Figure 3(d)) of tabun are doublets due to phosphorus couplings.

6.7 NMR Spectral Parameters of Selected CWC-related Chemicals

Molecular structures, NMR spectral parameters, and sample conditions for selected scheduled CW agents and some of their precursors and hydrolysis products are presented in Table 1. The NMR spectral parameters are given in CDCl_3 or D_2O , or both (sometimes at different pH values). Resonances of the chemicals can be assigned from the data, but the assignments are not alone sufficient for identifications.

7 DATABASES

In the following, we list the requirements for the construction of an in-house NMR spectrum library for verification of CWC-related chemicals, and briefly describe the OCAD and the VERIFY database (VERIFY's analytical reference database).

7.1 Requirements for an In-house Library

The main requirements for the construction of an in-house NMR spectral library for verification of CWC-related chemicals may be listed as follows:

- All ^1H , $^{13}\text{C}\{^1\text{H}\}$, ^{19}F , $^{31}\text{P}\{^1\text{H}\}$, and ^{31}P NMR spectra relevant to the molecular structure should be included (cf. Figures 2 and 3).
- Spectra should be recorded with the authentic chemical dissolved in a commonly used NMR solvent, for example, CDCl_3 or D_2O . Since the solvent, and the pH in aqueous samples, may affect the appearance of the spectrum, particularly in ^1H NMR, it is advisable to record spectra in different solvents (e.g. CD_2Cl_2 , acetone- d_6) and at different pH values (e.g. pH 0; pH approx. 7 or nonadjusted; pH 14).
- NMR spectral parameters that can be easily determined from the spectrum should be included, and the spectrum quality should be evaluated. The authentic chemical must be relatively pure so that there is no disturbing overlap of the resonances of the authentic chemical. In the evaluation of the spectra, the laboratory should follow the OPCW evaluation criteria (see Section 7.2).
- MS and/or IR spectroscopic analyses of the same authentic chemical should have been done to confirm the identity.

The principal contents of an NMR spectrum library page are (see Figures 2 and 3):

- spectrum type;
- full-width spectrum and sufficient expansions to reveal the spectral details;
- chemical information (name, CAS number, structure);
- NMR spectral parameters (chemical shifts and coupling constants);
- sample conditions, experimental conditions, and data-processing conditions;
- spectrum quality (line width) and traceability (source reference).

7.2 The OPCW Analytical Database

An important part of implementing the CWC is establishing reliable verification mechanisms. As the analysis of samples taken on the occasion of inspections is one part of this mechanism, the availability of collections of analytical data is essential. The OPCW, as the body responsible for the implementation of the CWC, wishes to have its own Analytical

Database, which contains as much data on CWC-related chemicals as possible. Since on-site analysis in particular by MS is planned for future verification activities, library of mass spectra is mandatory. Retention times of chemicals are collected for chromatography analyses. Although NMR spectroscopy is not suitable for on-site analysis, it is nevertheless considered an essential technique (as may be also IR) for laboratories specialized in the detection and identification of CW agents and related chemicals. Qualified laboratories from all parts of the world were therefore asked to submit their mass, NMR, and IR spectra and retention time data to be included in the OCAD.

An atlas of NMR spectra (the OCAD version 7 from April 2004 contained 1391 NMR spectra) of the OCAD has been compiled with the assistance of dedicated laboratories worldwide. The efforts of the laboratories and the OPCW have yielded a useful high-quality NMR spectral database. The only factor limiting its usefulness could be the difference of the instruments (in magnetic field strength and resonance frequency) that were used, because the resonance frequency may affect the spectrum appearance, in particular, in ^1H NMR. However, this is not considered to be a serious problem because many of the spectra were recorded on 300–400-MHz instruments whose spectra do not differ much from those recorded at 200 or 500 MHz. The difference between the two extremes may be larger. The OPCW requires that all spectra to be included in the OCAD be evaluated and validated.

On behalf of the OPCW, the submitted spectra were evaluated by groups of specialists. It was decided that only 1-D ^1H , $^{13}\text{C}\{^1\text{H}\}$, ^{19}F , ^{31}P , and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra recorded on FT spectrometers with a proton frequency of 200 MHz or higher should be included in the database. The spectra were evaluated according to the following main criteria:

- the spectrum must be consistent with the assigned structure;
- the name, CAS number (where available), and chemical structure with numbering of atoms must be indicated on the spectra;
- resonances must be assigned where reasonably possible;
- coupling constants must be included where easily extractable;
- expansions must be displayed where relevant;
- resonances of impurities must be marked with asterisks;
- resonances of impurities must not overlap the resonances of the chemical of interest;
- the solvent and pH (if relevant) must be specified;
- the reference chemical must be indicated;
- if the reference chemicals are other than TMS or TSPA- d_4 for ^1H , TMS for $^{13}\text{C}\{^1\text{H}\}$, external H_3PO_4 for ^{31}P , and CFCl_3 for ^{19}F set at 0 ppm, their chemical shifts must be specified;
- the spectrum must have been recorded with adequate resolution to enable all required information to be derived;
- the spectra must be phased correctly;
- the S/N must be adequate for the detection of all relevant signals;
- the type and frequency of the spectrometer must be indicated; and
- the spectral width must be indicated.

Even before the CWC entered into force, the group of specialists approved some 900 NMR spectra recorded from 205 chemicals. Originally, it was decided to compile the accepted spectra as paper copies; now the OCAD data is distributed on a DVD as pdf files. A first version of the resulting atlas of NMR spectra was certified in February 1998. The process of extending the OCAD, which is to be made available on request to member states, is in continuous progress.

7.3 VERIFY Database

VERIFY is a computerized database designed for the storage, management, and search of chemical and analytical reference data ⁽⁴⁴⁾. Properties and contents of the NMR part of VERIFY are:

- NMR spectrum;
- chemical information;
- spectrum identification code and spectrum type;
- molecular structure and numbering of nuclei;
- NMR spectral parameters (chemical shift and coupling constant data) for nuclei;
- sample and experimental conditions;
- instrument information;
- capability for display, expansion, and printing of spectra; importing and exporting of spectra

in JCAMP-DX format; no capability for spectral search.

The VERIFY is designed to work in the PC environment. The advantages of the computerized version relative to the paper version of the NMR library are easier access to the spectra and data search routines.

8 PERSPECTIVES AND FUTURE DEVELOPMENTS

8.1 LC/NMR

Liquid chromatography has been hyphenated on-line for the separation of analytes prior to their detection by NMR^(45–48). LC/NMR provides a means for almost background-free NMR spectra. The high-intensity signals from the elution system solvents and modifiers may overlap resonances of interest, but the most parts of the background are expected to be free from signals. Modern experimental solvent peak suppression methods help in decreasing the intensity of the unwanted high signals by allowing better use of the dynamic range but not solving the problem of partial overlapping. Basic detectors for LC are the ultraviolet (UV) and diode array detectors (DAD). The detection capability in LC/NMR applied for trace analysis of CWC-related chemicals is improved considerably if an MS can be used as the detector simultaneously. In an optimal case, a powerful analysis system is then formed, that is, LC/DAD/MS/NMR. In the system coupled with MS, 5 % of eluent flow is directed to the MS and the main flow goes to NMR. The best sensitivity in LC/NMR is obtained when running in stopped flow. In that mode, the chemicals of interest (in a LC peak) are stopped at the active area of the probe head. The diffusion can be considered negligible so that several NMR experiments can be recorded successively. The system described above provides UV, MS, and NMR data of the same chemical simultaneously.

8.2 Ultra-high-field Spectrometers

A 400-MHz spectrometer is today a mid-frequency instrument. With a higher-field spectrometer, the

detection limit is lower and the resonances are better dispersed, thus solving in part the problem of resonance overlap. By way of example, a ¹H sensitivity of 1234:1 (0.1 % ethylbenzene; see Section 4.4) was obtained on an 800-MHz spectrometer⁽⁴⁹⁾. This means that roughly one order of magnitude less chemical can be detected.

8.3 ‘Nano’ Probe Technology

Less than 1 µg quantities of analytes in about 50–60 µL of solvent can be detected with a novel probe head known as the *nanoprobe*⁽⁵⁰⁾. Although with this probe head the absolute amount of a chemical needed for a useful spectrum is low, the problem of resonance overlap remains.

9 COMPARISON WITH OTHER SPECTROMETRIC TECHNIQUES

The main advantages of NMR spectroscopy in the verification of CWC-related chemicals are as follows:

- At sufficient concentration and in the absence of disturbing background resonances, NMR is the superior method both for the identification of known chemicals and for the structural elucidation of unknown chemicals. Its usefulness in identification is attributable to the fingerprint nature of spectra, while the usefulness in structural elucidation rests on the structural specificity of the spectra. The wide variety of routine 1-D and 2-D experiments available is of assistance in both identification of chemicals and structure elucidation.
- Vast majority of the scheduled chemicals are directly analyzable by NMR: the spectra can be recorded in aqueous or in organic liquids from acidic, neutral, or basic chemicals, and from alkylated and protonated salts.
- Because NMR is a nondestructive method, the NMR samples can be used for the preparation of samples for other analytical techniques. (Note that in some cases the deuterated solvent may deuterate the chemicals of interest affecting their detection, for example by GC/MS.)

The main disadvantages of NMR are the following.

- There may be background resonances and, therefore, possible overlap of resonances of the chemicals of interest.
- There is no separation method for NMR comparable to GC for MS and Fourier Transform Infrared (FTIR) (see Section 8.1, however).
- Sensitivity is low: the detection limits are high compared to MS and FTIR.
- NMR is not suitable for on-site analysis.

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ABBREVIATIONS AND ACRONYMS

$^{13}\text{C}\{^1\text{H}\}$	Experiment: Carbon-13 Observation with Simultaneous ^1H Decoupling
^{19}F	Nucleus: Fluorine-19; Experiment: Fluorine-19 Observation
^1H	Nucleus: Hydrogen-1 or Proton; Experiment: Hydrogen-1 or Proton Observation
^{31}P	Nucleus: Phosphorus-31; Experiment: Phosphorus-31 Observation
$^{31}\text{P}\{^1\text{H}\}$	Experiment: Phosphorus-31 Observation with

1-D

2-D

ASC

ASTM

CAS

COSY

CWC

CW

DAD

FID

FTIR

FT

GA

GB

GC/FTIR

GC/MS

GC

GD

HD

HMQC/TOCSY

HMQC

HN-1

HPLC

HSQC

IEC

IR

ISO

LC/DAD/MS/NMR

Simultaneous ^1H

Decoupling

One-dimensional

Two-dimensional

Automatic Sample

Changer

American Society for

Testing and Materials

Chemical Abstracts

Service

Correlation Spectroscopy

Chemical Weapons

Convention

Chemical Warfare

Diode Array Detectors

Free Induction Decay

Fourier Transform Infrared

Fourier Transform

Tabun

Sarin

Gas

Chromatography/Fourier

Transform Infrared

Gas Chromatography/Mass

Spectrometry

Gas Chromatography

Soman

Mustard Gas

Heteronuclear Multiple

Quantum Coherence/Total

Correlation Spectroscopy

Heteronuclear Multiple

Quantum Coherence

Nitrogen Mustard

High-performance Liquid

Chromatography

Heteronuclear Single

Quantum Coherence

International

Electrotechnical

Commission

Infrared

International Standard

Organization

Liquid

Chromatography/Diode

Array Detector/Mass

Spectrometry/Nuclear

LC/NMR	Magnetic Resonance Spectroscopy
	Liquid Chromatography/Nuclear Magnetic Resonance Spectroscopy
LC	Liquid Chromatography
L	Lewisite-1
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
OCAD	Organization for the Prohibition of Chemical Weapons Central Analytical Database
OPCW	Organization for the Prohibition of Chemical Weapons
QC	Quality Control
Q	Sesquimustard Gas
TMS	Tetramethylsilane
TOCSY	Total Correlation Spectroscopy
TSPSA	3-trimethylsilyl)propane Sulfonic Acid Sodium Salt
T	Oxygen Mustard
UV	Ultraviolet
VERIFIN	Finnish Institute for Verification of the Chemical Weapons Convention
VERIFY	VERIFIN's Analytical Reference Database
VX	O-Ethyl S-2-diisopropylaminoethyl Methylphosphonothiolate

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CHAPTER 14

Fourier Transform Infrared Spectroscopy in Analysis of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Infrared spectroscopy (IR) is one of the oldest instrumental analytical techniques but its value in structural analysis has decreased with the rise of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Compared to the traditional dispersive IR techniques, Fourier transform infrared spectroscopy (FTIR) offers more sampling techniques.

In the analysis of environmental samples, chromatographic techniques are necessary as the sample matrices can be very complicated. The best commercially available IR technique for environmental

analysis, so far, is gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR). The combined use of GC/FTIR with different gas chromatography/mass spectrometry (GC/MS) methods gives reliable results in the analysis of environmental samples. The power of FTIR is that it gives very characteristic spectra, which can be used almost like fingerprints.

The Chemical Weapons Convention (CWC), which entered into force in April 1997, contains provisions for chemical analysis of samples in both on-site and off-site laboratories. On-site laboratories will be manned by the inspectors of the Organization for the Prohibition of the Chemical Weapons (OPCW) and equipped with the analytical equipment

approved by the States Parties to the OPCW. Off-site laboratories will analyze ambiguous samples, which the on-site analysis cannot resolve. These accredited testing laboratories are equipped with sensitive instruments and are designated by the OPCW on the basis of performance in proficiency tests.

There are some political issues related to the on-site analysis performed by the OPCW inspectors. One of these is the issue of assuring the confidentiality of commercial business information. There is a fear within the chemical industry that the inspectors may obtain knowledge on the critical production methods through detailed and nonrestricted analysis of their process samples. Even though all the inspectors are under the obligation to maintain confidentiality, this remains a problem. One answer is to restrict the scope of the analysis to cover only the chemicals related to the CWC, for example, by including only spectra relevant to the inspection in the instrument's database. However, if spectra are measured so that the analyst can see the spectra and print them, there always is a possibility that an experienced analyst identifies the chemical, even if its spectrum is not included in the instrument's database. In the mobile mass spectrometer, a so-called blinded software, Automated Mass Spectral Deconvolution and Identification System (AMDIS), is used by the OPCW to overcome this problem. This software has been developed in the National Institute of Standards and Technology (NIST) in the United States ⁽¹⁾. However, no such software has been developed for FTIR, so far. Owing to this question, OPCW does not intend to bring an FTIR spectrometer on an on-site inspection. Therefore, FTIR will only be used in off-site laboratories or during non-OPCW on-site analysis.

Chemical warfare agents (CWA) are classified into several categories, for example, nerve agents and vesicants. Tear gases are forbidden as a method of warfare but still allowed for riot control purposes. The CWC lists chemicals in three Schedules, which have been constructed on the basis of the toxicity of the chemicals, their industrial use, and historical usage as warfare agents. Schedule 1 consists of families of toxic chemicals, which have been developed, produced, stockpiled, or used as chemical weapons, for example, sarin and mustard gas. These chemicals have little industrial use. Schedule 3, on the other hand, consists of 17 single chemicals with

very large industrial use, for example, phosgene and triethanol amine.

There are challenges in the CWC-related analysis. Even though the schedules restrict the number of possible target chemicals, the theoretical number of chemicals is still over ten thousand, even hundreds of thousands. Naturally, the number of practically important chemicals is much smaller. The properties of the chemicals vary from solids to gases and from volatile to nonvolatile chemicals. In IR, the absorptivities vary considerably.

One challenge in the analysis of the CWA is the analysis of their precursors and degradation products, which are often nonvolatile. The CWA degrade, for example, hydrolyze or oxidize easily. Traditional IR sampling techniques, like KBr pellets and liquid cells are well suited for analysis of neat or concentrated nonvolatile chemicals. Environmental samples containing these kind of chemicals, however, normally require derivatization before GC/FTIR analysis.

In the field of CWC-related analysis, the chemicals should be identified by comparing their spectra to spectral libraries or to spectra measured from authentic chemicals. In infrared spectroscopy of CWC-related chemicals, the spectral interpretation is not enough for unambiguous identification, but it is an important tool in the structural elucidation of unknown chemicals.

In analytical chemistry, combination of results of several different analytical techniques gives the most reliable results. In a well-equipped off-site laboratory, results from FTIR, MS, and NMR, with other analytical data, can be combined to produce reliable, unambiguous analytical proof to support the chemical disarmament.

Some good books are available on some of the subjects discussed in this article, for example, on combination of chromatography and FTIR ⁽²⁾, FTIR spectroscopy in general ^(3,4), and IR spectra of phosphorus-containing chemicals ⁽⁵⁾.

2 SAMPLE PREPARATION

Many of the chemicals related to the CWC are very toxic and, therefore, care must be taken while handling samples that possibly contain these chemicals.

The different sample preparation or introduction methods in IR can be divided into two categories: methods in which no sample separation takes place and methods in which the sample undergoes separation prior to FTIR measurement. The former class, for example, KBr pellets, liquid or gas cells, and attenuated total reflectance (ATR), can be used only for relatively pure and concentrated samples, for example, bulk materials. The latter class, for example, gas chromatography (GC), is required when analyzing environmental samples or mixtures where the target chemical is not concentrated. The American Society of Testing and Materials (ASTM) has published a standard practice describing different general IR techniques ⁽⁶⁾.

There are restrictions in the selection of the sample preparation or introduction method when the laboratory is taken on site. Methods requiring supplies difficult to provide in field conditions, typically liquid nitrogen, cannot be applied on site. Thus, GC-based and ATR methods are not normally applicable on site. A suggestion for a basic on-site sample preparation kit is shown in Table 1.

The physical state of the chemical to be analyzed determines the sample preparation method. The majority of the chemicals listed in the Schedules of the CWC are liquids in room temperature. There are also gases or solids in each schedule. Some chemicals are borderline cases, which may be solids in room temperature but melt in the infrared beam, or liquids that are too volatile in the infrared beam. Table 2 summarizes some typical chemicals of each type in the Schedules.

Sample introduction using GC solves many problems related to the analysis of chemicals related to the CWC. The amount of chemicals needed is low, which almost removes the danger in handling these chemicals. A drawback in the GC is that it can only be used to analyze volatile chemicals. Volatile derivatives can be made of most of the nonvolatile scheduled chemicals.

2.1 KBr Pellets and Liquid Cell

Most solid or liquid chemicals related to the CWC can be analyzed using KBr pellets or liquid cells.

Table 1. A suggestion for a basic on-site FTIR kit

General equipment		
Sealed and desiccated FTIR spectrometer		
Evacuatable desiccator with desiccant		
Transport container for KBr pellets/powder with desiccant		
Small pump for evacuating desiccator and gas cells		
Pasteur pipettes and bulbs		
Forceps with replaceable rubber tubing around the tips		
Polishing kit: glass plate, felt pad, abrasive		
Solvents: acetone, dry ethanol		
Gloves (nonpowdered)		
Decontamination solution		
Containers for decontamination (for samples, tissue, and glass)		
Decontaminable trays for sample preparation		
Tissue and filter paper		
Small vials (e.g. 4 or 8 ml) with caps		
Chemical agent detector		
Glove bag/box or fume cupboard		
Outer container for liquid or solid samples		
Equipment for liquid samples	Equipment for solid samples	Equipment for gas samples
Liquid cell	KBr powder	Gas cell
KBr windows for liquid cell	Hydraulic or hand press	KBr disk for gas cell
Holder for liquid cell	KBr pressing kit (with spare steel pellets if possible)	Holder for gas cell
Disposable sample cards (option)	Mortar and pestle	Tubing and connectors as required
	Spatulas	Pressure sensor
	Mulling agent if required	Bottle for vaporization

Table 2. Summary of the physical states of some scheduled chemicals in the CWC. CAS numbers and melting/boiling points for borderline chemicals are shown in parenthesis

Physical state	Schedule 1	Schedule 2	Schedule 3
Solid	Salts of VX Saxitoxin (35523-89-8) Ricin (9009-86-3)	BZ (6581-06-2) Salts of nitrogen compounds	Phosphorus pentachloride (10026-13-8) —
Solid/Liquid	Bis(2-chloroethylthio)methane (63869-13-6, mp 6 °C)	Methylphosphonyl dichloride (676-97-1, mp 33 °C)	Triethanol amine (102-71-6, mp 21 °C)
Liquid	Sarin (107-44-8) Tabun (77-81-6) VX (50782-69-9) Mustard gas (505-60-2)	Dimethyl methylphosphonate (756-79-6) Thiodiglycol (111-48-8) —	Phosphorus oxychloride (10025-87-3) Trimethyl phosphite (121-45-9) —
Liquid/Gas	Chlorosarin (1445-76-7, bp 51 °C) Chlorosoman (7040-57-5, bp 65 °C)	—	Hydrogen cyanide (74-90-8, bp 26 °C) —
Gas	1,1-Dimethylethyl methylphosphonofluoridate (13273-12-6)	PFIB (382-21-8)	Phosgene (75-44-5) Cyanogen chloride (506-77-4)

The preparation of KBr pellets is a standard procedure in laboratory conditions since the hydraulic press used for pressing is considered standard equipment in an IR laboratory. In an on-site laboratory, the press is not a very practical piece of equipment as it weighs about 50 kg. If a press is a necessity on site, the possibility of using a handheld KBr press should be considered.

2.1.1 Solid Samples

There are several techniques to prepare solid samples for IR analysis: solid KBr pellets, melts, solutions, and mulls. While analyzing chemicals related to the CWC, introduction of additional absorbance bands in the spectra should be avoided. Therefore, the last two methods should not be used if other methods are available, since they always have peaks present because of solvent or oil.

Thorough instructions for preparing solid KBr samples are presented by Dent ⁽⁷⁾. He also describes trouble shooting for the preparation KBr disks.

When measuring spectra of very hard crystals, sometimes distorted bands, almost like in derivative spectra, can be seen with solid KBr or mulling techniques. This is caused by the Christiansen effect ⁽⁸⁾, which appears if too large crystals are left in the

sample after grinding. The infrared beam is reflected from the crystals, and as the refractive index changes over an infrared band, the peak shape is distorted. If the crystals are ground more so that the particle size becomes smaller than the wavelength of the infrared radiation (2.5 μm to 25 μm in the mid-IR region), no distortion appears. Figure 1 shows the Christiansen effect in the spectrum of tear gas CR, dibenz[b,f][1,4]oxazepine (CAS 257-07-8). First (Figure 1(a)), the sample grinding was deficient and the Christiansen effect was clearly visible. After this, the sample was extensively reground resulting in a nondistorted spectrum (Figure 1(b)). Atmospheric water has been introduced to the sample during the regrounding.

If no equipment for preparing the KBr disks is available on site, other methods must be chosen. Solid chemicals form a minority in CWC-related chemicals. These chemicals could be prepared on site, for example, by dissolving them first into a suitable solvent, depositing a couple of droplets on a KBr disk, and then evaporating the solvent off. The spectra obtained this way are not of the best quality, and it is not a normally recommended procedure in laboratory conditions, but often sufficient for identification as shown in Figure 1(c).

For comparison, a spectrum obtained by GC/FTIR (Figure 1(d)) is shown.

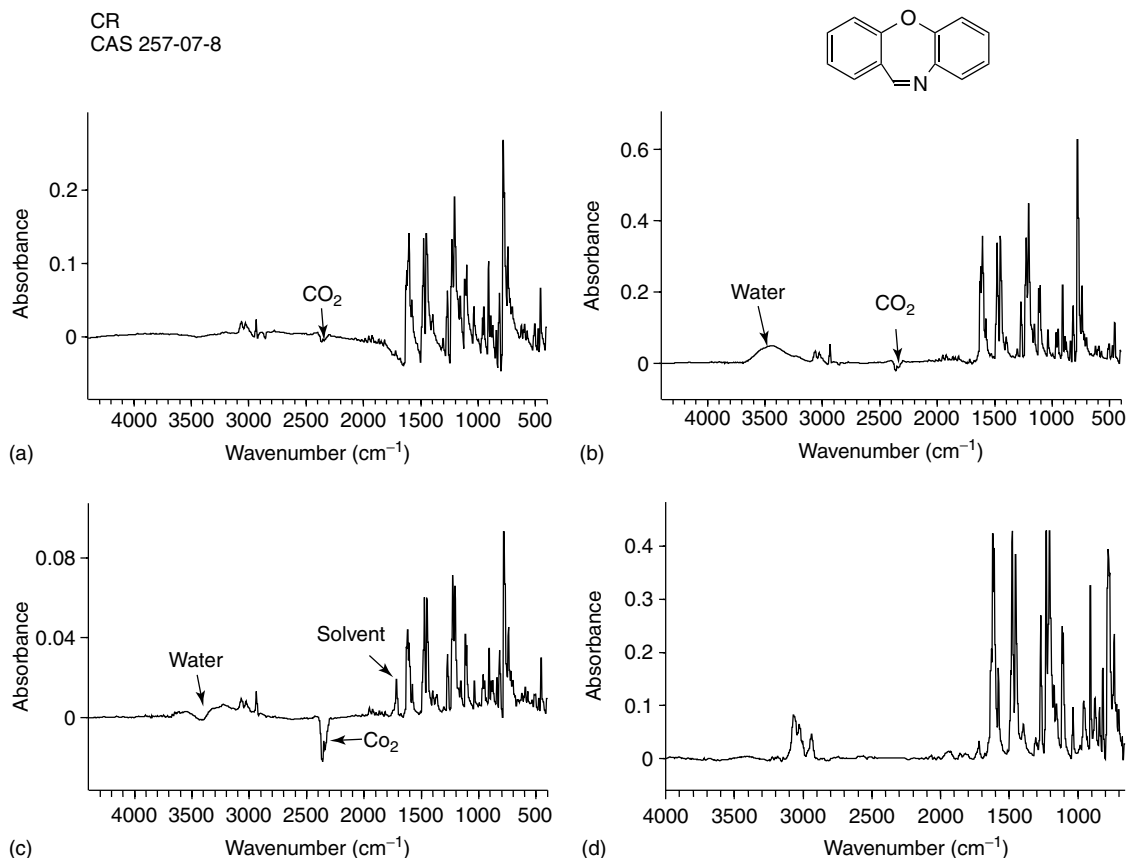


Figure 1. Spectra of CR using different sample preparation methods. The spectra have been measured (a) as a solid KBr disk with deficient grinding of CR (Christiansen effect visible), (b) as a solid KBr disk with CR well reground, (c) dissolving CR into acetone and deposition on a KBr disk, and (d) by cryodeposition GC/FTIR (Source: M. Söderström, unpublished results)

2.1.2 Liquid Samples

Generally, the analysis of pure liquid chemicals is very straightforward. Liquid chemicals with a boiling point below 100 °C should generally be analyzed in a sealed cell (preferable for CWC-related analysis) or in solution⁽³⁾. Other chemicals (bp > 100 °C) can be easily analyzed between KBr pellets. CWC-related chemicals are very toxic and therefore extra care should be taken in their analysis to avoid the vapor hazard.

Measuring the IR spectra of liquid chemicals is relatively simple, if commercial KBr windows for liquid cells are available. These windows can be reused if cleaned and decontaminated effectively. Decontamination solution containing

potassium hydroxide and ethanol works effectively, but it also corrodes the windows extensively. The decontaminated windows often need polishing to make them fully transparent and smooth again.

In cases where the windows have to be guaranteed to be clean, new windows should be used each time. However, this can become quite expensive if a large number of samples are to be analyzed. In such a case, the use of a press for preparing KBr pellets should be considered.

2.1.3 Safety

Always, when handling CWC-related chemicals, safety must come first. Sample preparation must

always be carried out in a fume cupboard. Protective gloves should be used always and a respirator should be kept available for emergencies. Work on live agents should not be performed alone; there should always be somebody who oversees the person handling the toxic samples.

An airtight sample holder or at least an outer container for the sample should be used when possible. The spectrometer can be placed in a fume cupboard to avoid vapor hazard, but good ventilation of the sample compartment can be enough. It should be remembered that no sample preparation should be carried out in the same fume cupboard to avoid contamination of the instrument. Decontamination of an FTIR spectrometer is very difficult if not impossible.

The work area should be kept tidy to enable safe sample preparation. It is a good idea to use trays in the fume cupboard. These are easy to clean and decontaminate after the work is finished and also, they contain any spillage of chemicals.

A good practice is to monitor the workspace inside the fume cupboard with a continuous handheld or tabletop chemical agent detector, if such a device is available. There are several models commercially available based on ion mobility, flame photometric, enzymatic, or photoacoustic detection ⁽⁹⁾.

2.2 Attenuated Total Reflectance

ATR is a technique based on total internal reflections at the crystal surface. The infrared spectrum is measured from a very thin volume surrounding the infrared transparent ATR crystal. This technique is one of the best IR sampling techniques suitable for analyses of chemicals in water. Detection limit is less than 1 mg/ml for nerve agents ⁽¹⁰⁾.

The sampling cell with the crystal can be also a flow-through cell, which makes the sample handling easier.

The use of an ATR accessory normally requires a Mercury–Cadmium–Telluride (MCT) detector to achieve enough sensitivity. The detector requires liquid nitrogen cooling, which limits the on-site usage of this method.

Normally, the biggest problem with analysis of CWA with nonchromatographic FTIR techniques is the large quantity of analyte needed for the analysis.

The analyzed chemical presents a danger to the analyst unless the sample is enclosed in an airtight container. The other way of solving the problem is to place the instrument in the 'hot' area and protect the user, for example, by protective clothing.

SensIR Technologies has taken the latter choice with their TravelIR HCI™ instrument, where HCI stands for 'Haz[ardous]Mat[erial] Chemical Identifier'. The instrument has been designed for working in 'hot' areas, keeping ease of use and decontamination in mind. Various hazardous material sampling teams in the United States have used the instrument. It was also taken into use by inspectors of United Nations Monitoring, Verification and Inspection Commission (UNMOVIC) in Iraq ⁽¹¹⁾. Often, the ATR accessory is used with an MCT detector, which requires liquid nitrogen cooling. The TravelIR uses a Peltier-cooled DLATGS (Deuterated L-Alanine Triglycine Sulfate) detector, which operates in normal room temperature, thus allowing easy portability. The detector is not as fast and sensitive as MCT, but it is sufficient for the ATR analysis of pure chemicals. The only requirement is power even though the instrument can be fitted with a battery pack. The weight of the instrument is 11.8 kg without the battery pack.

It should be noted that UNMOVIC was not governed by the same restrictions as OPCW in on-site FTIR sampling as discussed earlier.

2.3 Gas Cell

Gas cells can be used for gaseous and volatile liquid samples. The use of the gas cell is simple, but care should be taken in emptying the cell when the sample is toxic. A gas sample can be sucked in to an evacuated cell from the atmosphere or from a container containing gas, if suitable connection is available. Volatile liquid samples can be placed as droplets into the cell, which is closed, and the chemical is allowed to vaporize. The gas-phase spectrum can then be measured. The droplets can also be placed in a separate bottle for the vaporization, and the vapor can then be transferred into the evacuated gas cell.

The difficulty in gas sampling is that the spectra differ from the condensed phase spectra. Therefore, reference spectra of gas-phase spectra are required for accurate identifications.

In principle, the cell should not need cleaning, other than emptying it, after the analysis since only the gaseous phase is measured. However, one should be certain that all possible volatilizable chemicals are removed from the surfaces before the next measurement. The KBr windows of a gas cell may require polishing after use, especially when used in humid conditions.

2.4 Gas Chromatography

In general, GC is well suited for analysis of toxic volatile chemicals since the analyzed quantities can be low. Normal injection volumes with capillary columns are 1–2 μL . Most of the CWA are volatile, and their nonvolatile degradation products can be made volatile through derivatization (e.g. methylation or silylation).

There are three different types of GC/FTIR interfaces: light-pipe, matrix isolation (MI), and cryodeposition (also direct-deposition, cryotrapping). In the two latter techniques, the sample is deposited on a surface before measurement of spectra. All three techniques have been used for the analysis of CWC-related chemicals. Light-pipe interface has been the most popular, even though the usage of cryodeposition in this type of analysis has been increasing over the years.

One important factor to consider when selecting the type of GC/FTIR instrument is the availability of spectral libraries for each interface type. This issue is discussed in more detail below in Section 3.3.

In the field of FTIR, the availability of the instruments has a dualistic nature. Basic instruments, spectrometers, and basic sampling accessories, are available from multiple sources. The problem lies in selecting the right model from all available possibilities. In GC/FTIR instruments, the case is different. While the major FTIR manufacturers produce light-pipe interfaces, only few deposition-type instruments have been available. Erickson reviewed the situation of the GC/FTIR market in 1998 ⁽¹²⁾. The article discusses general developments and future prospects of GC/FTIR.

One of the simplest light-pipe GC/FTIR instruments is the Infrared Detector (IRD) from Digilab (formerly from Bio-Rad and before that from Hewlett-Packard). This instrument is a spectrometer and light-pipe built into a single device and optimized

to be used as a GC detector. This instrument is a common GC/IR/MS solution. It is also marketed as such. The problem with this type of hyphenation is that most part of the GC effluent is directed to the light-pipe, thus reducing the sensitivity of the MS.

The production of two deposition-type instruments have been ceased: the Cryolect[®] matrix isolation instrument from Mattson and the Tracer[®] cryodeposition instrument from Bio-Rad. Currently, two new instruments of these types are available: The Detective cryodeposition instrument from Bourne Scientific and the ClearIR[™] matrix isolation instrument from Reedy Scientific Instruments, Inc.

The Detective from Bourne Scientific includes both the deposition system and an ABB Bomem spectrometer. The interface can be modified to act as a detector for a GC, a high-performance liquid chromatography (HPLC), or a supercritical fluid chromatography (SFC). The chromatograph effluent is deposited on a rotating IR transparent ZnSe window. The tip is moved so that the sample is deposited in a spiral pattern. The interface contains a zero dead volume plumbing system, which can be used to divert the solvent peak away from the deposition system. Additionally, there is a dump/solvent sink on the edge of the ZnSe window. The whole system is placed in a vacuum chamber to prevent unwanted heat transfer in the system. The temperature of the ZnSe window is modified according to the chromatograph type. In GC mode, liquid nitrogen is used for cooling. According to the manufacturer, the sensitivity of the system is the same as that of the sensitivity of a Tracer. The adjustments of the deposition system have been made easier than that in the Tracer system.

Reedy Scientific sells a ClearIR[™] GC/IR/MS system, which splits the GC (Thermo Finnigan Trace GC) effluent between an IR spectrometer (Thermo Nicolet Nexus 670), with a matrix isolation interface, and mass spectrometer (Thermo Finnigan's PolarisQ[™] quadrupole ion trap or TRACE DSQ[™] single quadrupole instrument). The GC effluent is mixed with 2 % argon. The mixture is directed to the surface of a cryogenically cooled rotating cylinder.

From the OPCW proficiency tests, it can be seen that from the seventh OPCW proficiency test (spring 2000) onward, Tracer has been the only FTIR technique for which use has been reported. Four laboratories have used this technique. At least two other laboratories working in the field of CWC

analysis have used Tracer at some stage in CWC-related analysis. One laboratory has purchased the Detective from Bourne Scientific and tried in the OPCW proficiency tests without submitting any GC/FTIR data in the reports.

ASTM has published a standard practice for gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) analysis covering basic features of each type of interface ⁽¹³⁾.

2.4.1 Light-pipe

Light-pipe interface is the easiest method for connecting a GC and an FTIR spectrometer. The light-pipe is a long flow cell with reflective inner coating and IR transparent windows at both ends of the cell. It is very easy to operate and to maintain. The problem of light-pipe interface is that it is not very sensitive compared to a normal bench-top mass spectrometer, which makes the analysis of the same samples with both instruments difficult.

The lower sensitivity of the light-pipe system has to be compensated by either additional sample preparation (e.g. concentration of the sample) or injecting more sample using a large-volume injection system. Both these methods concentrate not only the sample but also the chemical background present in the sample.

The IR spectra of the chemicals are measured in the vapor phase, as the GC effluent is hot. One of the problems associated with the spectrum measurement in the gas phase is that a gas-phase spectrum of a chemical differs considerably from those measured in other phases. See Section 3.3 for further discussion on this subject.

2.4.2 Matrix Isolation

Matrix isolation as a GC detector was commercialized in the mid-1980s. The GC effluent is mixed normally with argon (ca. 2%) and then trapped on a rotating gold-coated cryogenic cylinder at about 12 K. The eluted molecules are isolated from each other in an argon matrix, which forms a solid track on the surface. Reflection-absorption spectra are collected from the surface after the trapping.

MI spectra have very narrow bands as there are no intermolecular interactions. Because of the narrower bands, less overlapping of the vibrations

occurs and more spectral details can be seen. MI spectra are different from all other types of spectra. Therefore, they cannot directly be compared with any other data. See Section 3.3 for further discussion on this subject.

2.4.3 Cryodeposition

Cryodeposition is the newest interface type for a GC/FTIR instrument. In this system, the eluents in the GC effluent are frozen on an IR transparent slide, which is cooled using liquid nitrogen. The carrier gas evaporates in the process so that the chemicals are directly deposited on the slide surface. Transmission spectra are then measured through the slide. These spectra are like normal condensed phase spectra, with rare exceptions. The sensitivity is five times better than in light-pipe, and the same or even slightly better than in GC/MI/FTIR.

Since the cryodeposition instrument is based on depositing the eluting compounds in very small spots (ca. $100 \times 100 \mu\text{m}$) on the depositing surface, all large amounts deposited on the surfaces will interfere with the operation of the system by spreading too widely on the surface. For this reason, the eluent exiting the deposition tip must be directed to a solvent sink (i.e. away from the slide) until the solvent has fully eluted. This necessary solvent delay in starting the data collection results in missing all the chemicals eluting until the solvent or any other large peaks (e.g. the silylation reagent peaks) have eluted.

2.4.4 Comparison of Interfaces

Using either GC/MI/FTIR or cryodeposition, GC/FTIR spectra should be obtained from the same environmental samples (1–15 ng of chemical) as in GC/MS, either without additional sample preparation or with slight concentration. The chromatograms produced by GC/FTIR instruments differ somewhat from those in GC/MS because in IR, the absorbance varies greatly depending on the chemicals. However, the same chromatographic peaks can be located in the different chromatograms.

GC resolution degrades slightly in all of the available GC/FTIR interfaces. In the light-pipe type interface, some broadening of the chromatographic peaks occur because the diameter of the light-pipe

(often 1 mm) is larger than that of a capillary column (0.25–0.5 mm). Thus, the GC resolution degrades by an average factor of 1.2⁽²⁾. In the MI and cryodeposition interfaces, there is spreading of the chromatographic peaks due to the deposition of the sample on a surface, causing the GC resolution to decrease by an average factor of 1.8⁽²⁾.

As already mentioned above, it is possible to hyphenate GC/MS and GC/FTIR instruments so that two analysis results can be obtained with one injection. This type of system is cost-effective, but from the analytical point of view is not always recommendable. Neither of the instruments can be used fully. If the GC/FTIR interface is of the light-pipe type, the concentration requirements are not met with most of the chemicals. In some instruments, the GC effluent is split 1 to 10 between MS and FTIR. The result of the hyphenation can be that the sensitivity of the MS is lowered 10 times, but the concentration of the chemical is still too low for FTIR identification.

The speed and sensitivity of the measurement requires an MCT detector to be used in a GC/FTIR instrument. Additionally, matrix isolation and cryodeposition techniques use at least liquid nitrogen to enable the trapping of the eluents. The need for cold liquids renders the GC/FTIR methods logistically difficult.

2.4.5 Derivatization

The most common derivatization methods in the analysis of chemicals related to the CWC are methylation with diazomethane (CAS 334-88-3) and silylation with either *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; CAS 25561-30-2) or *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA; CAS 77377-52-7). The former method changes hydroxyls of acid groups to methoxy groups and the latter method changes hydrogens of hydroxyls to trimethylsilyl (TMS) or *tert*-butyldimethylsilyl (TBDMS) groups⁽¹⁴⁾. Figure 2 demonstrates the effect of the two derivatization methods (diazomethylation and silylation with BSTFA) on methylphosphonic acid (CAS 993-13-5).

The advantages of methylation are that the sizes of the derivatized chemicals do not increase very much and the dissolved derivatization reagent does

not change the chromatogram very much. One of the disadvantages is that this method does not derivatize alcohols, which sometimes are difficult to analyze with nonpolar columns like SE-54 (5 %-diphenyl-95 %-dimethylsiloxane), which is commonly used in this type of analysis. Additionally, the group added to the molecule, methyl, is a naturally occurring group. It is impossible to know from just the methylated sample if the chemical in it originally contained the hydroxyl or methoxy version of the chemical. For confirmation of this, the analysis of underivatized or silylated sample is also required.

Silylation results in a more complicated sample than methylation, but it also shifts the derivatized chemicals to longer retention times. As the TMS group does not come from natural sources, the original chemical in a sample can be deduced without comparison to an untreated sample. Silylation enhances retention characteristics of chemicals much better than methylation. It also silylates active sites of the column material. However, when starting to analyze silylated samples with the cryodeposition system, the solvent delay time must be increased to avoid deposition of the large amount of early eluting residue of the derivatization reagent present in the sample after the reaction. This results in loss of chemicals eluting simultaneously with the reagent.

Analysis of lewisites, Lewisite 1 (CAS 541-25-3) and lewisite 2 (CAS 40334-69-8), also requires derivatization. Normally, either 3,4-dimercaptotoluene (DMT; CAS 496-74-2) derivative or 1-butanethiol (CAS 109-79-5) derivative is made. Analysis of an underivatized lewisite can be harmful to the GC columns normally used.

Before an injection of a derivatized sample, care must be taken to ensure that the injection system is clean from derivatizable chemicals from previous injections. They might be derivatized by the derivatization reagent and elute, giving false positive results. A good way to check the cleanness of the system is to inject 1–2 µl of the derivatization reagent solution.

2.5 Other Techniques

There are a few liquid chromatography/Fourier transform infrared spectroscopy (LC/FTIR) instruments commercially available, but there are so far no published applications on the analysis of chemicals

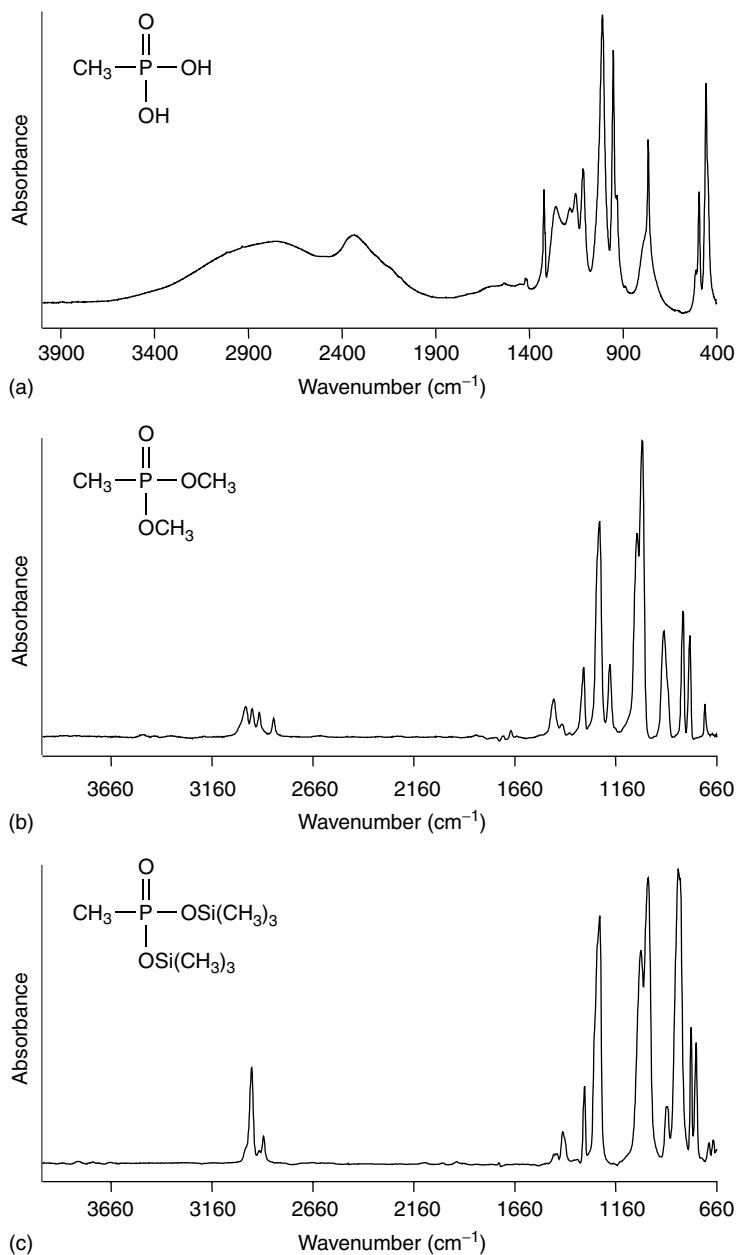


Figure 2. The effect of derivatization on methylphosphonic acid: (a) methyl phosphonic acid analyzed as solid KBr pellet, (b) dimethyl methylphosphonate analyzed with cryodeposition-FTIR, and (c) bis(trimethylsilyl) methylphosphonate (CAS 18279-83-9) analyzed with cryodeposition-FTIR (*Source*: M. Söderström, unpublished results)

related to the CWC. The analysis of water-soluble degradation products would be a suitable area for this method. It would not be logistically viable to take an LC/FTIR instrument on site.

Infrared microscopy, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, and photoacoustic spectroscopy (PAS) techniques may be suitable for some types of sample but the use of

these methods for analyses of CWA has been not been reported.

Promising studies have been published on the use of remote detection of CWA, but they are outside the scope of this article.

3 DATA EVALUATION

When IR is used for identification of chemicals from CWC-related samples, it is important to verify or at least compare the results with those from different analytical techniques. Especially, when environmental samples are analyzed using GC/FTIR, results should be compared with results from GC with selective detectors and GC/MS. All these techniques are powerful in chemical identification when used together in a collaborative manner. This requires the use of similar GC conditions and columns. It has been a standard practice to require consistent results from at least two different analytical methods to confirm unequivocal identification. These methods can include among others GC retention index monitoring, gas chromatography/electron ionization/mass spectrometry (GC/EI/MS), gas chromatography/electron ionization/high-resolution mass spectrometry (GC/EI/HRMS), gas chromatography/chemical ionization/mass spectrometry (GC/CI/MS), gas chromatography/chemical ionization/tandem mass spectrometry (GC/CI/MS/MS), GC/FTIR, and NMR (^1H , $^{13}\text{C}\{^1\text{H}\}$, ^{19}F , ^{31}P , or $^{31}\text{P}\{^1\text{H}\}$).

After the analysis itself, there are several steps to the final result. In GC/FTIR, the peaks of the analytes of interest must be located from the chromatogram. The chemicals must then be identified by comparison with reference data. If this is not possible, the spectra should be interpreted so that reference material can be either obtained or synthesized.

3.1 Gram–Schmidt Chromatogram

In GC/FTIR, the chromatogram representing the absorbance of each scanset versus retention time is called the *Gram–Schmidt chromatogram* (GSC). The GSC resembles the total ion chromatogram (TIC) in MS and a flame-ionization detector (FID) chromatogram. It is not a simple sum of all

absorbances of a spectrum, but calculated using interferograms as vectors⁽¹⁵⁾. This technique is faster than first performing a Fourier transform and then integrating the spectrum. Normally in GC/FTIR, the interval between consecutive data points has to be about one second to achieve the necessary chromatographic resolution. This one second left for the calculation of a data point in a chromatogram used to be beyond the capabilities of the computer.

The GSC differs from a TIC in MS or a FID chromatogram in GC. The relative intensities of the chromatographic peaks are sometimes very different in IR due to differences in absorptivities between chemicals. For example, chemicals containing phosphorus–oxygen bonds have high absorptivities. Additionally, as the chromatographic resolution is lower in GC/FTIR, closely eluting peaks may overlap in IR, but not in MS or GC.

Software of some instruments uses a threshold for storing of GC/FTIR data. This means that only data on peaks above a set threshold are stored. This approach is not generally recommended for analyses of environmental samples when the concentration of the target analytes is not known in advance. It is important to be able to examine the spectra of minor components also.

3.2 Functional Group Monitoring

A useful practical procedure in GC/FTIR is functional group monitoring. During the data collection or, in some systems, just after it, absorbance of certain selected spectral regions in each spectrum is plotted against the retention time to produce new chromatograms, functional group chromatograms (FGC). This enables the chromatographic peaks of interest to be selected in an easier way than by using the GSC.

Many chemicals related to the CWC contain functional groups that give rise to strong, characteristic absorption bands. These are summarized in Table 3.

The use of functional group monitoring will make it possible or at least easier to find the target chemicals in the midst of background chemicals. For example, very probable background chemicals in samples from a battlefield or industry could be those from gasoline, diesel fuel, rubber, and paint or even humic acids. These chemicals can mask

Table 3. Summary of the most characteristic functional groups of the chemicals related to the CWC. Many of these groups can be used as a basis for functional group chromatograms. Wavenumber regions are based on data from M. Söderström, unpublished results and ⁽⁵⁰⁾

Chemical group	Functional group	Region (cm ⁻¹)	Strength
Nerve agents and their degradation products	–P=O and –P–O–C–	1350–990	s to vs
Sulfur and nitrogen mustards, aminoethylchlorides	–C–Cl	720–660	s
Degradation products of sulfur and nitrogen mustards	–C–OH	3400–3200 ^a 3643–3630 ^b	vs m
Lewisites, BZ and its degradation products, tear gases	=C–H	3150–3000	
BZ and its degradation products, some tear gases	–C=O	1750–1700	vs
Trimethylsilyl derivatives of hydroxyl-containing degradation products	–C–O–Si(CH ₃) ₃ –P–O–Si(CH ₃) ₃	1110–1080 1156–975 852–847	 vs vs
Ethers and esters	–C–O–	1300–1000	vs
All	–C–H	3000–2800	s
Many of precursor chemicals and degradation products	–O–H	3400–3200 ^a 3643–3600 ^b	m m
	–N–H	3398–3381 3344–3324	w w
Aminoethylthiols	–S–H	2950–2550	w
BZ and its degradation products, tear gases	=C–H Ring	3100–3000 2000–1660 1600–1450 1225–1000 770–735	w w w w s to vs

^aintermolecular hydrogen bonded.

^bfree OH (i.e. very low concentration or vapor phase).

the target chemicals by overlapping and make the sample too complex to be able to find the relevant chemicals in the GSC. Figure 3 shows an example of an extract of a rubber sample containing two mustard agents. In the functional group chromatogram 750–680 cm⁻¹, the peaks of the two agents can be seen clearly.

3.3 Reference Spectra and the Spectral Libraries

In the FTIR analysis of the chemicals related to the CWC, the spectral libraries are essential. For the purposes of the CWC, there has to be a reference spectrum for identification. If there is no infrared reference spectrum available, the identification cannot be accepted unless a reference

compound can be synthesized and a reference spectrum measured.

3.3.1 Available Reference Data

There are no commercial libraries available that contain more than few occasional CWC-related chemicals. Therefore, each laboratory has been forced to create its own infrared libraries.

The OPCW has collected reference infrared as well as mass and NMR spectra, since 1994. First, a hard copy set of these spectra was made available for States Parties to the Convention, that is, countries that have ratified the CWC, in 1999. At the time of writing this article, in August 2004, the 7th version of the OPCW Central Analytical Database (OCAD) has been released. It used to be distributed on a CD, but as the size of the database has been growing it

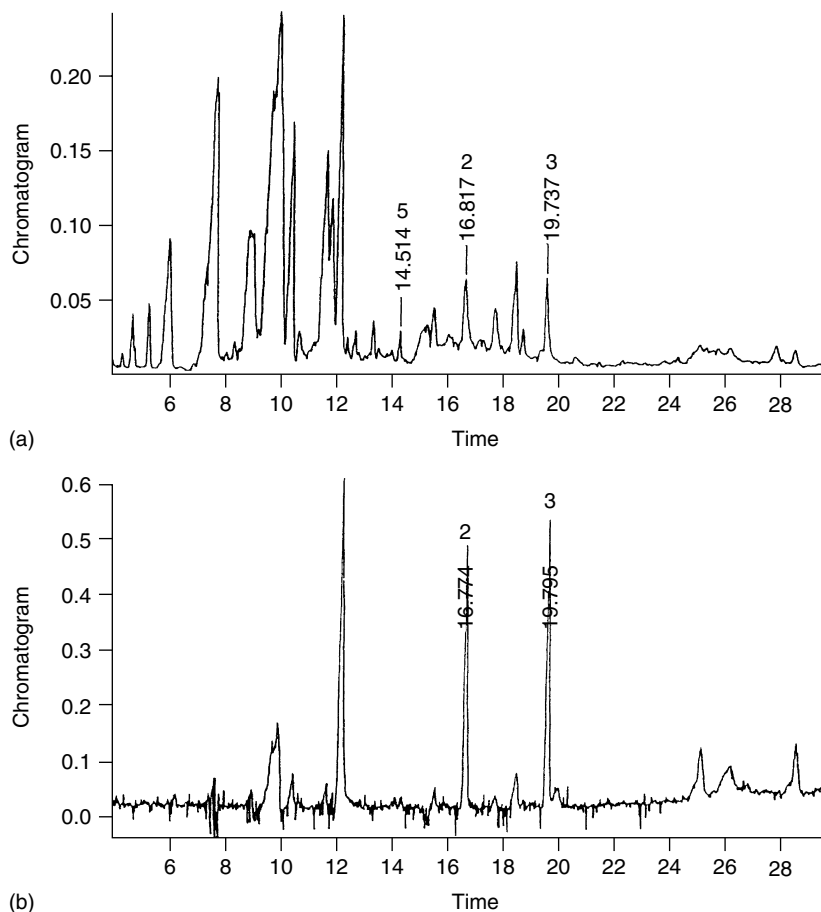


Figure 3. Chromatograms of an extract of a rubber sample containing two mustard agents (peaks marked with 2 and 3): (a) Gram–Schmidt chromatogram and (b) functional group chromatogram 750–680 cm⁻¹ (Reproduced by Permission of the Finnish Ministry for Foreign Affairs from International Interlaboratory Comparison (Round-Robin) Test for the Verification of the Chemical Disarmament, F.3. Testing of Procedures on Simulated Industry Samples, The Ministry for Foreign Affairs of Finland, Helsinki, Finland, 1992, ref. 18)

will be distributed on a DVD. OCAD consists of a hard copy library (PDF-OCAD) and an electronic library (e-OCAD). The version 7 released in April 2004 (PDF-OCAD v.7 and e-OCAD v.5) contains 2603 mass spectra, 710 infrared spectra, 1391 NMR spectra (¹H, ¹³C, ¹³C{¹H}, ¹⁹F, ³¹P, and ³¹P{¹H}), and 3078 GC retention indices. All mass spectra are available both as hard copy files and electronic spectrum files. Other data is available only as hard copy files. There are some plans to make the infrared data available also as electronic spectrum files. The infrared data has been measured using five different methods: cryodeposition GC/FTIR (ca 70 % of the

spectra), light-pipe GC/FTIR (ca. 15 %), liquid KBr or cell (ca. 10 %), solid KBr (ca. 1.5 %), and gas cell (ca. 0.5 %).

Some infrared spectra of chemicals related to the CWC can be found in open literature. The largest collection of spectra can be found in two of the so-called Blue Books of the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN) published in 1977 and 1982 ^(16,17). Also, Blue Books describing the results of international interlaboratory comparison tests contain some example spectra ^(18–22). Shagidullin *et al.* ⁽²³⁾ have published an IR atlas of organophosphorus

chemicals that contains spectra of some chemicals relevant to the CWC. Several articles contain IR spectra or spectral data for nerve agents and related chemicals^(24–31) and vesicant-related chemicals^(32–34).

3.3.2 Spectral Differences Due to Sample Introduction

The phases of the chemicals measured in light-pipe, matrix isolation, and cryodeposition instruments are different: vapor phase, matrix-isolated, and condensed phase respectively. The intermolecular interactions are missing in the vapor phase and matrix isolation. Therefore, for example, all hydrogen bond-related vibrations are missing or different. Also, the vibration bands are narrow in the gas phase and even narrower in matrix isolation. Thus, the spectra cannot be compared with each other. The traditional IR spectra measured using salt pellets or windows produce also condensed phase spectra, which are therefore comparable with cryodeposition spectra (see example in Figure 4). There are other differences because of factors of more practical nature: lower sensitivity and resolution. Owing to all these differences, separate sets of reference spectra have to be measured for each interface type.

The light-pipe spectra are usually measured at the resolution of 8 cm^{-1} with four coadded scans, whereas the cryodeposition are often rescanned to a resolution of 4 cm^{-1} with 64 to 512 scans. Figure 5

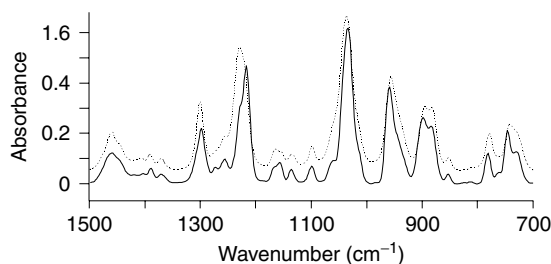


Figure 4. Part of cryodeposition (solid line) and liquid phase (dotted line) spectra of *O*-ethyl *S*-[2-(dimethylamino)ethyl] methylphosphonate (Reproduced by Permission of American Institute of Physics from M.T. Söderström, in de Haseth (ed.) *Fourier Transform Spectroscopy*: 11th International Conference, American Institute of Physics, New York, USA, 1998, pp. 457–460, ref. 28)

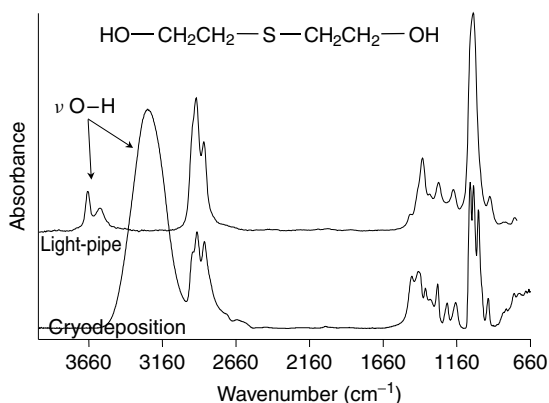


Figure 5. Spectra of thiodiglycol: top – light-pipe spectrum at 8 cm^{-1} ; bottom – cryodeposition spectrum at 4 cm^{-1} (Source: M. Söderström, unpublished results)

shows light-pipe and cryodeposition spectra of thiodiglycol. MI spectra differ considerably from both light-pipe and cryodeposition spectra since the molecules are normally isolated molecules in a noble gas matrix at very low temperatures. Thus, the spectral peaks are very sharp due to loss of intermolecular interactions and low rotation in the molecules. Like cryodeposition spectra, MI spectra can be measured with a higher number of scan and resolution.

Norton *et al.* studied the use of cryodeposition spectra for searching in condensed phase libraries⁽³⁵⁾. They analyzed six barbiturates using both KBr and cryodeposition GC/FTIR. They could identify all barbiturates using KBr disk spectra as a reference for cryodeposition spectra. The search results were better when only the region below 2000 cm^{-1} was used in the search. Norton *et al.* explain that highly polar compounds with C_2 symmetry can deposit on a crystalline substrate (such as KBr), as highly oriented crystal does, making the spectrum different. ZnSe is amorphous and the deposition process is so fast that no orientation can take place.

About 70% of the IR spectra collected by the OPCW are in a condensed phase. The largest commercial condensed phase infrared library (Sadler Condensed Phase IR Standards library) has over 75 000 spectra, while the largest vapor phase library (Sadler Vapor Phase IR Standards library) has less than 10 000 spectra. There are no commercial MI libraries available.

3.3.3 *Quality of Reference Spectra*

To make the identification of chemicals unambiguous, criteria for acceptable reference spectra are needed. All reference spectra should be evaluated using these criteria. The OPCW rules require that at least three evaluators agree on approval of the spectrum before it is accepted. The evaluation criteria defined by the OPCW are the following:

- the spectrum must be consistent with molecular structure of the chemical;
- the spectrum must show the absence of extraneous spectral features attributable to impurities or contaminants;
- the sample preparation method for the measurement must be stated;
- the minimum resolution must be 4 cm^{-1} for the condensed phase and normal gas-phase spectra, and 8 cm^{-1} for GC light-pipe spectra;
- the minimum spectral range must be at least $3700\text{--}700\text{ cm}^{-1}$ (for light-pipe spectra the limit can be $3700\text{--}750\text{ cm}^{-1}$);
- the signal-to-noise ratio must be adequate to detect all relevant peaks;
- the largest absorbing peak must not be saturated;
- the spectra must be adequately compensated for the atmospheric carbon dioxide and water;
- the intensity of the bands arising from water contained in the sample should be less than 5 % of the highest intensity absorption band of the sample; and
- for inclusion in the central OPCW Analytical Database, a spectrum must fulfill at least one of the following criteria:
 - it is consistent with IR data of the same chemical from another source; and
 - it is supported by accepted NMR or MS spectra obtained from the same sample with consistent results.

For the submitted reference spectra, the OPCW requires that the following data be attached to the spectra:

- OPCW code for the spectrum (assigned by the OPCW);
- Contributor's name and address;
- Contributor's signature;
- Chemical information;
 - Chemical name;
 - CAS registry number (if available);
 - Chemical structure;
 - Molecular formula (optional);
- Sample information;
 - Sample purity (optional);
 - Source (optional);
 - Sample phase;
- Instrument information;
 - Type (Fourier, grating, or prism);
 - Manufacturer;
 - Model;
 - Data system (optional);
 - Software version (optional);
- Experimental information;
 - Sampling mode (liquid, solid, solution, gas, light-pipe, cryodeposition, bulk, micro);
 - Measurement mode (transmission, absorbance, reflectance);
 - Baseline correction (manual, automatic, no);
 - Matrix;
 - Detector (optional);
 - Wavenumber range;
 - Indication of the ordinate scale;
 - Resolution;
 - Number of scans (optional); and
 - Date of experiment (optional).

3.4 Library Search

Searching the spectrum of an unknown chemical against a spectral library is a routine method used to identify chemicals. Most of the commercial infrared instruments include library search software that has several search algorithms to choose from. The search algorithm can sometimes have a strong effect on the library search result. This is due to the different ways the actual comparison between the spectra is done. Especially when the library and the unknown spectra have been measured differently (e.g. using solid KBr disk and cryodeposition GC/FTIR), the

IR band shapes and positions may vary slightly. In these cases, the choice of a proper search algorithm is essential.

Two of these search algorithms are least-squares metric (also called Euclidean) and derivative least-squares metric (also called derivative Euclidean) ⁽³⁶⁾. In both algorithms, the unknown and library spectra are normalized before the comparison. In the least-squares metric algorithm, the intensity difference between the corresponding data points in the spectra is raised to the second power. The sum of these differences is the Hit Quality Index (HQI). The derivative least-squares metric algorithm is based on the difference in the intensity change between two consecutive data points instead of the intensity of each point. There are many ways to apply the algorithms and to present the HQI, so it is impossible to give any estimates for a value for a good library match. The derivative least-squares metric algorithm is much more sensitive to changes in band position, which means that it should be used when the phase of the unknown and the library spectra are the same.

Figure 6 demonstrates an infrared library search. The unknown chemical was dimethyl ethylphosphonate (DMEP), measured using cryodeposition GC/FTIR. The library was a cryodeposition spectrum library. If the derivative least-squares metric algorithm (HQI can be 0–999; 999 is perfect match) is used, the result is DMEP (HQI = 558), dimethyl propylphosphonate (DMPP) (HQI = 275), dimethyl isopropylphosphonate (DMIP) (HQI = 246), and dimethyl methylphosphonate (DMMP) (HQI = 216) as shown in the Figure 6. The least-squares metric search gives a slightly different result: DMEP (HQI = 861), DMPP (HQI = 744), DMMP (HQI = 632), and DMIP (HQI = 607). It can be noticed that the first case gives much better indication of the correctness of the search result, by giving a bigger difference between the first and the second hit.

The search result should never be accepted on the basis of just the result listing, but should always be verified by an expert. It is therefore necessary for the analyst to have at least a basic knowledge on which spectral features to regard. When the analyst must differentiate between very closely related chemicals,

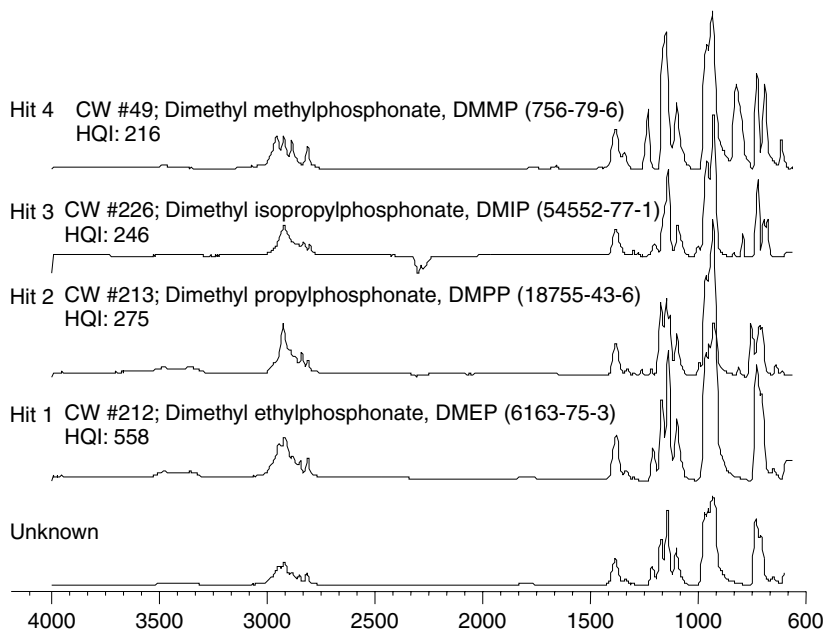


Figure 6. An example of a library search. An unknown chemical was searched against a library containing chemicals related to the CWC. The first hit, dimethyl ethylphosphonate was clearly the best candidate and matches the unknown. The search algorithm was a square derivative and the maximum HQI value is 999. (Source: M. Söderström, unpublished results)

for example, nerve agent homologues, more knowledge on the particular chemicals and the origin of the spectral bands is a prerequisite.

Identification chemicals from a spectra of mixtures is always somewhat difficult. Spectral subtraction can be sometimes used to reduce the complexity of the spectrum by removing the features of the subtracted spectrum from it. Subtraction seldom gives clean spectra, but it can at least help on the way to the final identification.

3.5 Interpretation

For the purposes of the CWC, results obtained with two spectrometric techniques are required for the identification of a compound. Although very useful as such, the interpretation of infrared spectra does not identify the chemical but may give tentative structures. Interpretation gives excellent results in some cases, but it should be remembered that the accuracy of interpretation depends on the type of the chemical and the experience of the scientist. In a case when an unknown compound is encountered, spectral interpretation (MS, IR, and NMR) is required so that the reference chemical can then be synthesized.

In the interpretation of infrared spectra of chemicals related to the CWC, it should be remembered that for some chemicals, for example, nerve agents, a structure could be proposed easily and accurately. For some other types of chemicals, for example, mustards and other vesicants, only the chemical class can be proposed. This is due to the fact that some functional groups do not have good structure specific group frequencies. Such spectra can only be used as a fingerprint for the chemical.

3.5.1 Phosphorus-containing Chemicals

3.5.1.1 Fermi Resonance In some compounds, a normally quite weak overtone or combination band may have almost the same frequency as another fundamental vibration. As a result, Fermi resonance may occur, that is, two relatively strong bands may be observed instead of the expected one band. The vibrations involved should be in the same part of the molecule so that they can be coupled. This effect can be found in at least some cryodeposition spectra of sarin and VX homologues.

The original positions of the fundamental and the overtone/combination bands can be calculated from the spectra using the formula (1) given by J. Overend⁽³⁷⁾, where ν_1 and ν_2 are the observed band frequencies and ρ is the intensity ratio of the bands.

$$\bar{\nu} = \frac{\bar{\nu}_1 + \bar{\nu}_2}{2} \pm \frac{\bar{\nu}_1 - \bar{\nu}_2}{2} \left(\frac{\rho - 1}{\rho + 1} \right) \quad (1)$$

3.5.1.2 P=O Thomas and Chittenden^(5,38-44) have carried out a thorough series of studies on the identification of organophosphorus compounds. The group frequency tables in these studies enable interpretation of many of the characteristic features in the spectra of nerve agents and related chemicals. Several structure-spectrum relationships give specific information on the molecule. One very valuable relation discovered by Thomas is the dependence of the position of the P=O bond stretching vibration, $\nu_{\text{P=O}}$, on the substituents on the phosphorus, represented by π constants:

$$\nu_{\text{P=O}} = 930 + 40\Sigma\pi \quad (2)$$

The π constants have been determined experimentally from over 900 compounds for a wide variety of substituents (91 substituents). Table 4 summarizes the most important groups that are present in the chemicals relevant to the CWC and their π values. Most of the calculated values are within $\pm 12 \text{ cm}^{-1}$ from the experimental values. For example, for sarin the calculated value is 1280 cm^{-1} , while the experimental values are 1277 cm^{-1} and 1272 cm^{-1} for liquid and cryodeposition spectra respectively.

Nyquist⁽⁴⁵⁾ states that the $\nu_{\text{P=O}}$ frequencies occur at higher wavenumbers in the vapor phase than in the condensed phase. He has modified the π -constant values in (1) to better match the slightly different band positions in the gas-phase spectra. These values are shown in Table 4 together with those for the condensed phase.

Cooper *et al.*⁽⁴⁶⁾ measured $\nu_{\text{P=O}}$ frequencies for dialkyl alkylphosphonates with a light-pipe GC/FTIR and found them to be those calculated using the π -constant values modified by Nyquist⁽⁴⁵⁾. They also discovered that the $\nu_{\text{P=O}}$ was in the region $1259-1248 \text{ cm}^{-1}$ in dialkyl isopropylphosphonates and in the region $1273-1265 \text{ cm}^{-1}$ in dialkyl *n*-propylphosphonates.

Table 4. Summary of values of π constant (see Equation 1) for the most important substituents present in chemicals relevant to the CWC (see Thomas ⁽⁵⁾ for condensed phase values and Nyquist ⁽⁴⁵⁾ for vapor phase values)

Group	π	
	Condensed	Vapor
CH ₃	2.1	—
CH ₂	2.0	—
CH	1.8	—
C	2.1	—
R	—	2.43
O—CH ₃	2.9	—
O—CH ₂	2.85	—
O—CH	2.75	—
O—R	—	3
O—SiR ₃	3.0	—
C≡N	3.5	—
S—alkyl	2.4	—
NR ₂	2.4	—
Cl	3.4	3.3
F	3.9	4.05

3.5.1.3 P-Alkyl The alkyl groups connected to phosphorus or to oxygen next to phosphorus can be identified in many cases. One of the most characteristic bands is the P—Me deformation near 1300 cm⁻¹ ⁽⁵⁾. There is also the P—Me rocking band in the region 934–900 cm⁻¹ ⁽⁵⁾. The bands of the other P-alkyl group do not give characteristic bands but can be identified by comparison with spectra of similar chemicals ^(28,47).

McGarvey *et al.* ⁽⁴⁸⁾ reported that in light-pipe GC/FTIR spectra, the region for P-methyl rocking in alkyl alkylphosphonofluoridates can be found in the region 928–917 cm⁻¹.

In cryodeposition, most of the studied 34 spectra show splitting of this band due to Fermi resonance. The region for the unsplit bands is 925–913 cm⁻¹, which is at a slightly lower position than in the gas phase. The regions for the split bands are 948–922 and 918–905 cm⁻¹. Using formula (1), the corrected band positions are found in the region 931–914 cm⁻¹, which make the overall region for this band 931–913 cm⁻¹.

Kireev *et al.* ⁽⁴⁹⁾, McGarvey *et al.* ⁽⁴⁸⁾, and Cooper *et al.* ⁽⁴⁶⁾ have published patterns characteristic for P-alkyl groups in the gas-phase spectra. Kireev (measured using a light-pipe GC/FTIR) reported

patterns for all alkyl groups. McGarvey (using a 10-meter gas cell at 50 °C) reported patterns for P-methyl and P-ethyl. Cooper (measured using a light-pipe GC/FTIR) reported patterns for P-propyl and P-isopropyl. Cooper gives regions for C—H deformations close to the P=O stretch, which can be used to differentiate P-propyl and P-isopropyl in dialkyl alkylphosphonates, alkyl alkylphosphonochloridates, and alkylphosphonofluoridates.

3.5.1.4 P—O—Alkyl Bands for P—O—Me (near 1180 cm⁻¹) and P—O—*i*-Pr (three bands 1100–1200 cm⁻¹) are characteristic ⁽⁵⁾. P—O—alkyl vibrations can also be identified by comparing patterns produced by known groups to those in the unknown spectrum ⁽⁴⁷⁾. A summary of some of these P—O—alkyl group patterns is shown in Figure 7.

3.5.1.5 P—F In the spectra of alkyl alkylphosphonofluoridates, there is always the distinct P—F stretching band. The range given by Thomas ⁽⁵⁾ for this vibration in sarin analogues (measured in a condensed phase) is 858–833 cm⁻¹. He reports the range 849–833 cm⁻¹ for P-methyl and 858–841 cm⁻¹ for other P-alkyls.

McGarvey *et al.* ⁽⁴⁸⁾ give ranges measured in the gas-phase spectra using a light-pipe GC/FTIR for P—F stretching in compounds containing different P-alkyl groups (at least 24 compounds in each group). In P-methyl-containing chemicals, the P—F vibrations occur in range 846–840 cm⁻¹, while the regions for P-ethyl, P-propyl, and P-isopropyl are 866–853 cm⁻¹, 870–853 cm⁻¹, and 865–847 cm⁻¹ respectively. The P-methyl region does not overlap with any of the other three and can be used diagnostically.

Unpublished results by Söderström, measured using a cryodeposition GC/FTIR, gave a similar range: P-methyl 844–837 cm⁻¹ (34 compounds), P-ethyl 868–829 cm⁻¹ (28 compounds), P-propyl 864–858 cm⁻¹ (26 compounds), and P-isopropyl 854–839 cm⁻¹ (19 compounds). The wide ranges of P-ethyl and P-isopropyl are due to the strong peak splitting in the region. In P—Et compounds, the range for compounds with unsplit bands is 850–842 cm⁻¹. For the split bands, the ranges are 868–849 cm⁻¹ and 843–829 cm⁻¹ for the upper and lower bands respectively. It seems, that Fermi resonance splits the P—F band. The original band

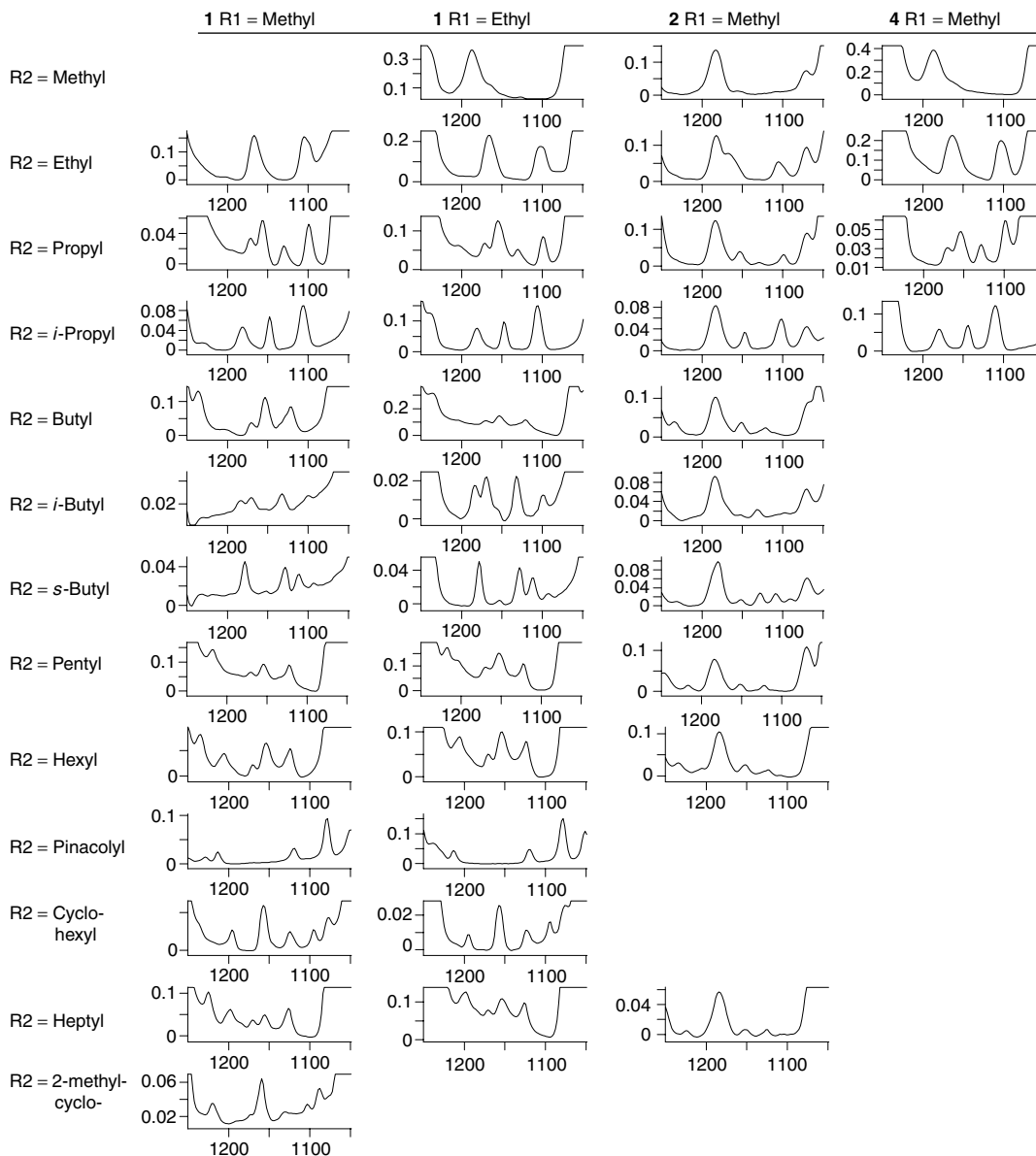


Figure 7. A summary of some P–O–alkyl groups. R¹ is P-alkyl and R² is P-O-Alkyl. The compared compounds belong to the sarin (**1**; alkyl alkylphosphonofluoridates), the tabun (**2**; dialkylamino alkyl phosphoramidocyanidates), and the dialkyl methylphosphonate (**4**) families. There are two peaks (1185–1180 cm⁻¹ and 1072–1070 cm⁻¹) in the tabun family (**2**) spectra in addition to the C–O peaks. These peaks are due to the C–N bond. The dialkyl methylphosphonate compounds (**4**) listed here are symmetric (R² = R²) (Reproduced by Permission of Springer-Verlag from M. T. Söderström, R. A. Ketola, *Fresenius J. Anal. Chem.*, 350, 162–167 (1994), ref. 49)

calculated from the spectra using formula (1) gives the range $852\text{--}840\text{ cm}^{-1}$ for the P–F vibration. This matches well with the range for compounds with unsplit bands given above. Similarly, the unsplit band for P-isopropyl gives the range $842\text{--}837\text{ cm}^{-1}$, while the split bands give ranges $870\text{--}845\text{ cm}^{-1}$ and $830\text{--}817\text{ cm}^{-1}$. The calculated range from the split bands is $846\text{--}834\text{ cm}^{-1}$. The overall ranges adjusted for Fermi resonance are $852\text{--}840\text{ cm}^{-1}$ for P-ethyl compounds and $846\text{--}834\text{ cm}^{-1}$ for P-isopropyl compounds. The diagnostic use of this band is difficult in cryodeposition due to the effect of the Fermi resonance. Only if an unsplit band is found in the region $864\text{--}858\text{ cm}^{-1}$, a P-propyl compound could be suspected.

3.5.1.6 N-Alkyl Some of the chemicals related to the CWC contain tertiary amine groups. The type of the alkyl groups (methyl, ethyl, propyl, or isopropyl) can be deduced in some cases from the stretching of the C–H bond next to the nitrogen atom. The value of the asymmetric stretching is lowered because of the interaction with lone pair electrons of the nitrogen atom⁽⁵⁰⁾, so that the band of this vibration is separated from the normal C–H stretching bands. Figure 8 shows some typical peaks of this kind in the VX-type chemicals⁽²⁸⁾.

3.5.1.7 $\text{C}\equiv\text{N}$ One very characteristic peak for tabun related chemicals is the stretching of the $\text{C}\equiv\text{N}$ bond. A sharp band due to this vibration can be seen at $2200 \pm 2\text{ cm}^{-1}$ ⁽⁴⁷⁾. There is always a band in the spectra of tabun homologues due to the P–N–Me group near 1320 cm^{-1} ⁽⁵⁾. This band occurs very close to the P–Me deformation band in, for example, sarin homologues. In the tabun homologues, however, the peak near 900 cm^{-1} due to the P–Me rocking is missing.

3.5.1.8 Interpretation Process Often, IR spectra can be only partially interpreted. An example of interpretation of the spectrum of sarin is shown in Figure 9. In the interpretation of a spectrum of an organophosphorus chemical, it is important to first deduce the type of the chemical. In the presented spectrum, the position of the $\text{P}=\text{O}$ stretching band (1272 cm^{-1}) is typical of sarin type chemicals. A very strong band typical for P–O–C is present at 1020 cm^{-1} . When there is also a peak matching the P–F stretching at 838 cm^{-1} , as well as the P–Me

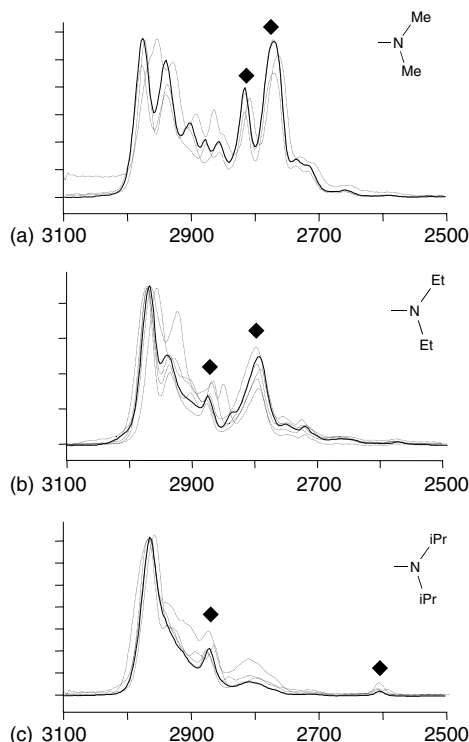


Figure 8. Typical bands (marked with ♦) due to the asymmetric C–H next to a nitrogen atom in some VX type chemicals: chemicals containing group (a) S-[2-(dimethylamino)ethyl], (b) S-[2-(diethylamino)ethyl], and (c) S-[2-(diisopropylamino)ethyl]. Spectra of several VX type chemicals are shown overlaid. (Reproduced by Permission of American Institute of Physics from M.T. Söderström, in de Haseth (ed.) *Fourier Transform Spectroscopy*: 11th International Conference, American Institute of Physics, New York, USA, 1998, pp. 457–460, ref. 30)

peaks at 1322 cm^{-1} and 927 cm^{-1} , the chemical seems to be an alkyl methylphosphonofluoridate. As the peak pattern in the region $1250\text{--}1050\text{ cm}^{-1}$ matches the P–O–isopropyl patterns shown in Figure 7, the proposal of the structure is isopropyl methylphosphonofluoridate, that is, sarin.

In the analysis of chemicals related to the CWC, spectral interpretation is not done with only one technique. Often MS or NMR spectra are used together with IR spectra in the elucidation of the structure of an unknown chemical. There are some examples of this type of elucidation available in the literature^(5,25,26,51).

One must remember that there are important spectral bands that do not fit in the detector range

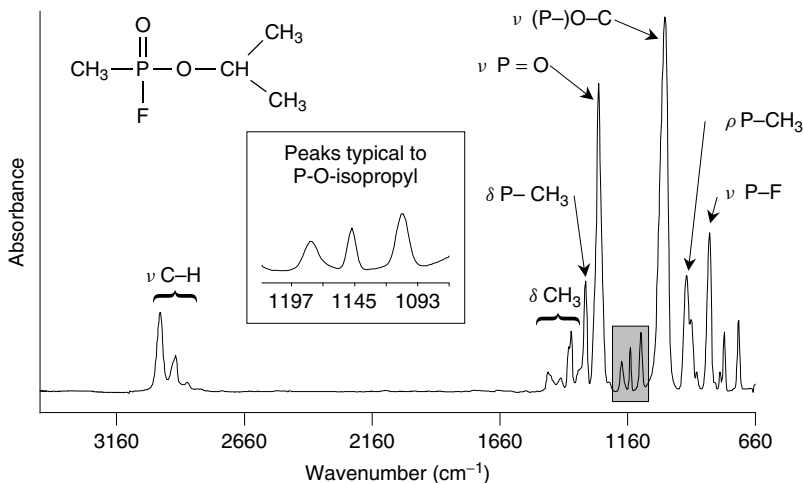


Figure 9. A partially interpreted cryodeposition spectrum of sarin (Source: M. Söderström, unpublished results)

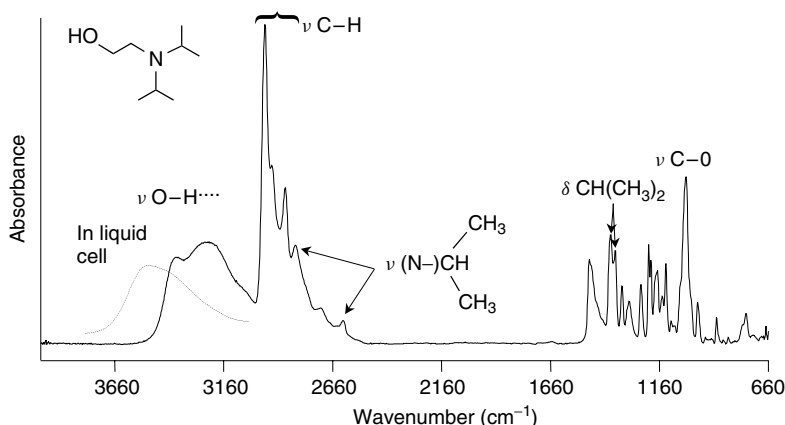


Figure 10. A partially interpreted cryodeposition spectrum of 2-(diisopropylamino)ethanol (CAS 96-80-0). Additionally, the O-H stretching band in a spectrum measured using a liquid cell is shown (Source: M. Söderström, unpublished results)

(e.g. $4000\text{--}700\text{ cm}^{-1}$) of a GC/FTIR instrument with a MCT detector. In CWC-related organophosphorus chemicals, for example, the bands due to P-S-C and P-Cl bonds are at too low frequencies to be seen in the spectra. The P-S-(C) frequency is $573\text{--}520\text{ cm}^{-1}$ in a VX-type chemical, and the general P-Cl frequency is $587\text{--}420\text{ cm}^{-1}$ ⁽⁵⁾.

3.5.2 Non-phosphorus Chemicals

Non-phosphorus chemicals related to the CWC are more difficult to interpret. There are functional

groups with characteristic vibrations in many chemicals, but the structure cannot be elucidated fully by interpreting the IR spectra as is the case with, for example, nerve agents. Table 3 contains some of the characteristic vibrations in non-phosphorus-containing CWC-related chemicals.

A partially interpreted cryodeposition spectrum of 2-(diisopropylamino)ethanol (CAS 96-80-0) is presented in Figure 10. In this case, the full structure cannot be deduced from the spectrum. There are two bands indicating the presence of a C-OH group in the molecule: stretching of the

C—O bond at 1035 cm^{-1} and the double peak of the hydrogen bonded O—H stretching at 3375 and 3230 cm^{-1} . In the Tracer spectra, the hydroxyl stretching band seems to appear as two bands in the symmetric dialkylaminoethanols. In asymmetric dialkylaminoethanols and in liquid phase spectra (band shown in Figure 10), the vibration appears as one band. The presence of an isopropyl can be detected from the presence of two bands at 2875 and 2607 cm^{-1} and two bands at 1382 and 1361 cm^{-1} . The former peaks are due to the (N—)C—H stretching like in VX-type chemicals (shown in Figure 8), and the latter peaks are due to the methyl stretchings in the $-\text{CH}(\text{CH}_3)_2$ group. The lack of other characteristic bands points to the presence of a scheduled chemical 2-(diisopropylamino)ethanol. Further confirmation for this is, however, required with other techniques.

3.6 Examples of GC/FTIR Spectra

Some of the most common chemical warfare and riot control agents are presented in Figure 11. Both light-pipe and cryodeposition type spectra are presented.

The chemicals in Figure 11 are nerve agents (sarin, soman, cyclohexylsarin, tabun, and VX), mustard agents (mustard and O-mustard), nitrogen mustard agents (HN-1, HN-2, and HN-3), and tear gases (CR and CS).

One can notice the effect of difference in the measurement conditions. Light-pipe spectra measured in gas phase are noisier than the cryodeposition spectra measured in the condensed phase, and the resolution is lower. Therefore, it is more difficult to see the fine structure of the spectra, which gives useful information for interpretation in the cryodeposition spectra.

One difference between the IRD and Tracer instruments is the spectral range, which in Tracer extends down to 660 cm^{-1} , while in IRD it extends only to 750 cm^{-1} . In practice, this results in cutting off some bands from the low-wavenumber end of the spectrum. Effects of this can be observed in spectra of mustard, O-mustard, HN-1, HN-2, and HN-3, which all contain a C—Cl group. The effect of this is most dramatic in the mustard spectra where the strongest band in the spectrum is eliminated, which will certainly have effect on the detection of this

chemical in chromatograms, especially in the FGC as the C—Cl stretching frequency cannot be utilized.

4 APPLICATIONS

The number of recent articles published on the FTIR analysis of the CWC-related chemicals is relatively low. Most of the research is old or unpublished.

4.1 International Tests

One of the best tests for methods and competence in the field of CWC-related analysis has been the international tests organized first by VERIFIN and later by the OPCW. Twenty tests have been held during 1989–2004 ^(18–22,52–66). FTIR has been used by few laboratories in the tests, as the laboratories with less resources have been concentrating on GC/MS analysis. The maximum number of GC/FTIR instruments used in the tests has been eight out of 26 laboratories. On average, laboratories using cryodeposition GC/FTIR instruments have performed better GC/FTIR analysis. In the beginning, some laboratories used nonhyphenated FTIR instruments but the results were poor when analyzing environmental samples. It should be noted that in the interlaboratory comparison test in 1991 ⁽²⁰⁾, FTIR was used successfully without GC separation. The test samples were not environmental but organic solution samples for which FTIR is suited well.

The international tests have shown that a GC/FTIR instrument is not a necessity for successful testing because two other techniques (GC/EI/MS, GC/CI/MS, NMR, or even GC retention index monitoring) can be used as the required different techniques. The major advantages of GC/FTIR are that it gives information on different characteristics of a chemical than, for example, MS and NMR. Therefore, it can make the overall spectral elucidation easier and faster. Also, the first ‘guesses’, that is, candidate structures for synthesis are more accurate when FTIR is used (see ref. ⁽²⁵⁾ for examples). The latter advantage is emphasized when analyzing phosphorus chemicals.

In the international tests, false identifications have occurred on the basis of misinterpretation of MS or NMR spectra only; for example, isopropyl methyl ethylphosphonate (CAS 141968-53-8) has

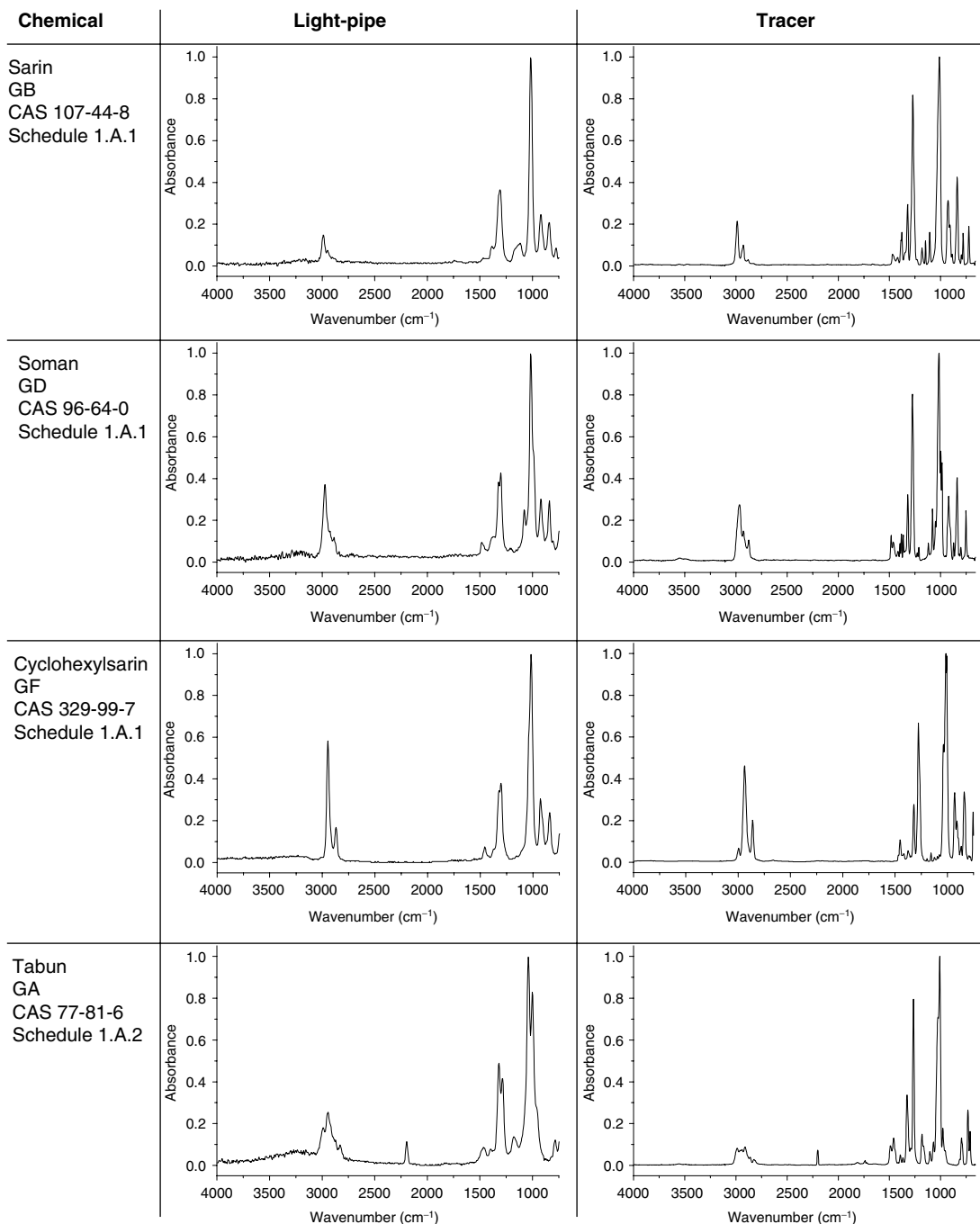


Figure 11. Light-pipe (using Hewlett-Packard IRD instrument) and cryodeposition (using Bio-Rad Tracer instrument) spectra for most common nerve agents, vesicants and riot control agents. All the presented spectra are normalized to absorbance value 1. (Light-pipe spectra © Crown Copyright Dstl 2004.)

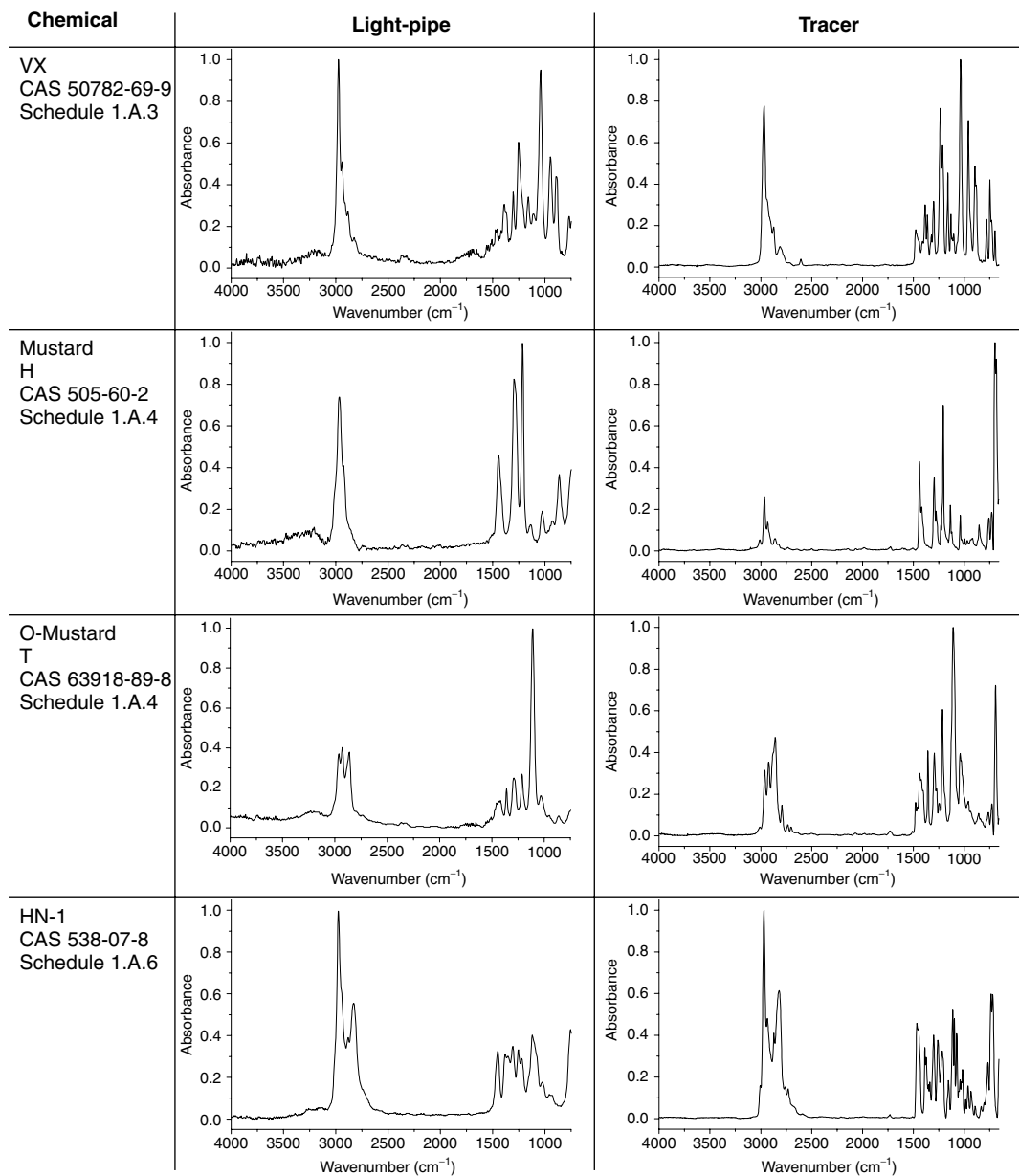


Figure 11. (Continued)

been identified instead of methyl propyl ethylphosphonate (CAS 170082-59-4) ⁽⁵³⁾, and methyl propyl ethylphosphonate instead of the methylation product of butylphosphonic acid (i.e. dimethyl butylphosphonate; CAS 24475-23-8) ⁽²²⁾. These mistakes were due to matching molecular weight, similarity of the

spectra, and the lack of reference data and could have been avoided by using additional techniques, such as GC/FTIR.

There are of course limitations in the interpretation capability with some chemical types. For example, spectra of mustard gas type chemicals

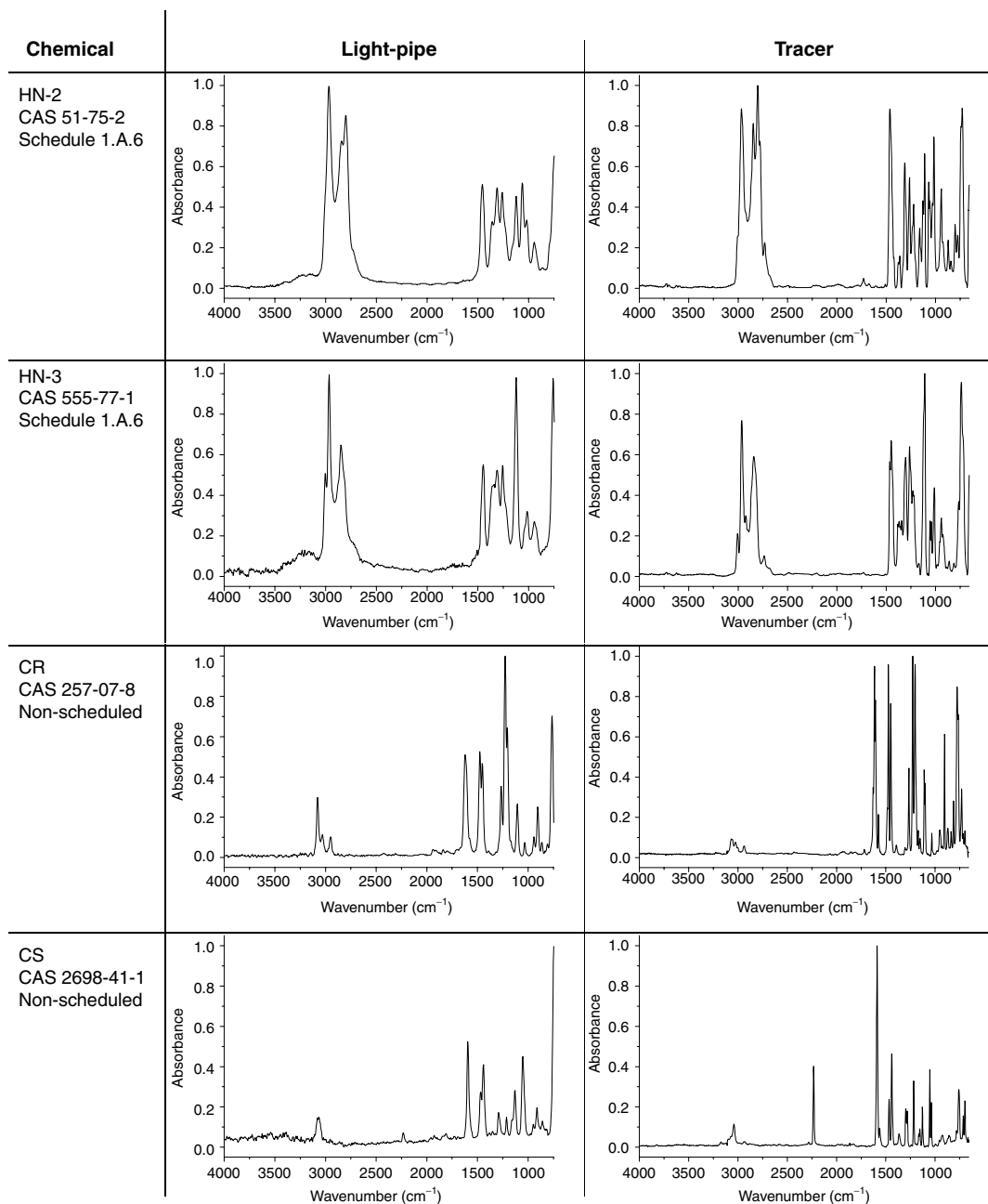


Figure 11. (Continued)

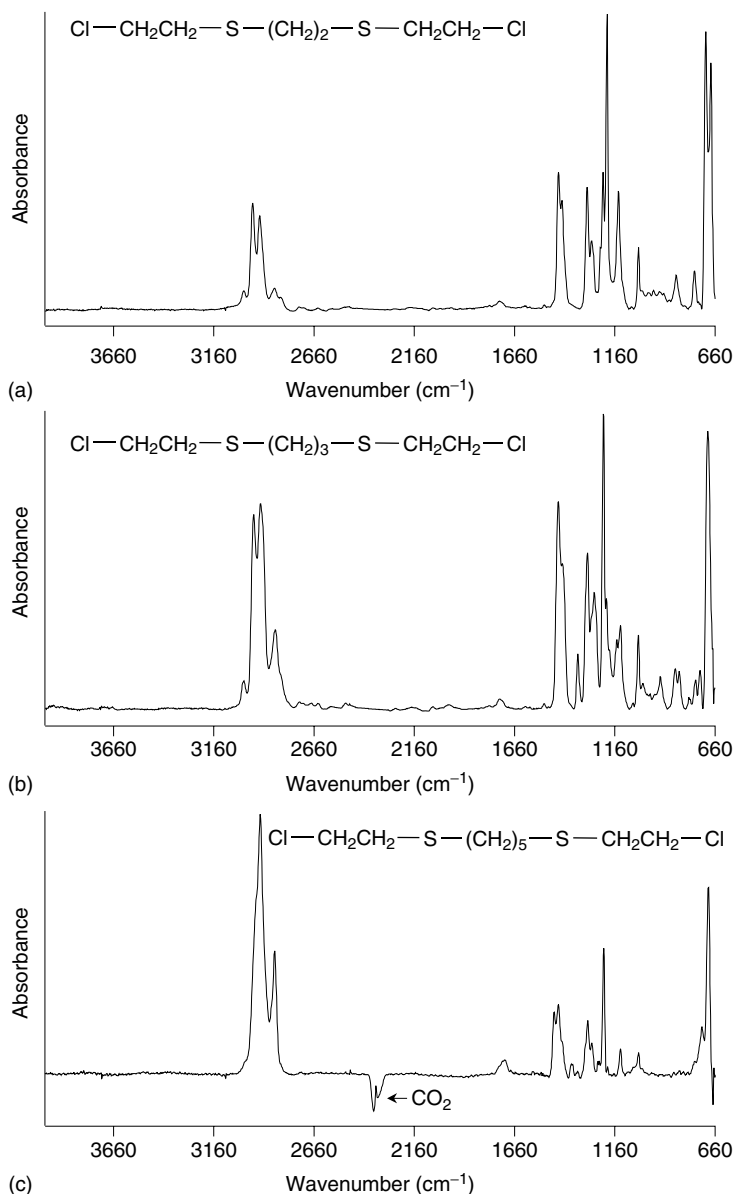


Figure 12. Cryodeposition-FTIR spectra of (a) sesquimustard (CAS 3563-36-8), (b) 1,3-bis[(2-chloroethyl)thio]propane (CAS 63905-10-2), and (c) 1,5-bis[(2-chloroethyl)thio]butane (CAS 142868-94-8) (*Source*: M. Söderström, unpublished results)

cannot be interpreted to produce an unambiguous analysis result. In the first official OPCW proficiency test ⁽⁵²⁾, there was a sample where three mustard type chemicals (spectra shown in Figure 12) were present; sesquimustard (CAS 3563-36-8), 1,3-bis[(2-chloroethyl)thio]propane (CAS 63905-10-2), and

1,5-bis[(2-chloroethyl)thio]butane (CAS 142868-94-8). In this case, reference spectra existed only for the first two compounds. The identity of the third could not be proven (only inferred) as a reference spectrum was not available. The spectrum and retention time definitely indicated the correct

answer, but they are not enough for an unambiguous identification.

4.2 ATR Applications

Braue and Pannella⁽¹⁰⁾ used a flow-through ATR cell for quantitative measurement of tabun, sarin, and soman in water. The sample change and the measurement could be performed safely due to quite dilute samples and airtight fittings. The method they developed has a useful range of 0.5–2.0 mg/ml. The quantitation is based on the P=O stretching near 1240 cm⁻¹.

Parchment *et al.*⁽⁶⁷⁾ used a diamond ATR probe to monitor decontamination of mustard gas, sarin, soman, and VX. They conclude that because of relatively low sensitivity of the method, it can only be used for analysis of CWA in realistic concentrations in real time and *in situ*.

The breakthrough of mustard gas and oxygen mustard (CAS 63918-89-8) in different types of rubbers⁽⁶⁸⁾ and polypropylenes⁽⁶⁹⁾ has been studied using ATR as one of the analysis techniques. One side of the material was exposed with the agents, while the unexposed side was against the ATR crystal.

4.3 GC/FTIR Applications

Both Söderström *et al.*⁽²⁵⁾ and Creasy *et al.*⁽²⁶⁾ have applied GC/FTIR together with several other hyphenated chromatographic techniques to analyze CWC-related chemicals in complex matrices. The results of the different technique have been combined to unequivocally identify the relevant chemicals in low concentrations. In both studies, IR and MS spectra have been used together in spectral interpretation. Weimaster *et al.*⁽⁷⁰⁾ studied samples collected in Iraq, using a very wide variety of instrumentation, but they could not find any scheduled chemicals.

Danian *et al.*⁽²⁷⁾ report structural identification of a VX type chemical using GC/MS, NMR, and GC/FTIR. They conclude that the identification of the chemical based only on MS data is very difficult.

The use of NMR and FTIR data makes the identification more reliable.

Durst *et al.*⁽²⁹⁾ made microscale synthesis of several sarin analogues in autosampler vials and analyzed them directly with a gas chromatography/infrared spectroscope/mass spectrometer (GC/IR/MS) instrument. Ninety different alkyl and cycloalkyl alcohols were used. They presented a couple of light-pipe IR spectra and a table of typical gas-phase frequencies ($\nu_{\text{P-F}}$, $\nu_{\text{P=O}}$, $\rho_{\text{P-methyl}}$, $\delta_{\text{s P-methyl}}$, $\nu_{\text{P-O-C}}$) for 12 sarin-type chemicals.

Sokołowski and Szymańska⁽³⁰⁾ analyzed tabun, which had been stored for at least 20 years, with a GC/IR/MS instrument. First, the sample was analysed without any derivatisation. Portions of it were then methylated or silylated and analyzed. They reported nine light-pipe IR spectra of derivatized degradation products.

Goeringer and Ellzy⁽³¹⁾ used GC/MI/FTIR to analyze alkylphosphonic acids from simulated river water samples. The chemicals were silylated before the analysis.

Smith *et al.*⁽³⁴⁾ studied three isomers (*cis*-, *trans*-, and geminal) of a lewisite using GC/MS, GC/FTIR, and NMR.

Söderström *et al.*^(47,71) studied the interpretation of spectra of some organophosphorus chemicals, as well as their IR and retention index searches. Some example spectra, chromatograms, and interpretations are given.

Brickhouse *et al.*⁽⁷²⁾ used a GC/IR/MS instrument, among others to characterize the contents of a munitions shell that was found. As suspected, the contents contained phosphorus chemicals used as simulants and did not contain any CWA.

Several publications describe studies of phosphorus-containing CWA using light-pipe GC/FTIR. The results of the spectral analyses have been discussed in the spectral interpretation section above. McGarvey *et al.* synthesized many sarin family analogs (at least 24 different ester groups for each P-alkyl)⁽⁴⁸⁾. The synthesis was performed as laid out by Durst *et al.*⁽⁷³⁾ The synthesized chemicals were analyzed using Bio-Rad IRD. The measured spectra were used for studying the characteristic vibrations in the spectra of sarin type chemicals. Cooper *et al.*⁽⁴⁶⁾ studied 10 alkylphosphonofluoridates and the respective alkylphosphonochloridates also using the IRD.

Both Kireev *et al.* ⁽⁴⁹⁾ and Cooper *et al.* ⁽⁴⁶⁾ have studied dialkyl alkylphosphonates also using a Hewlett-Packard (currently Digilab) IRD instrument.

5 QUALITY CONTROL

One aspect in the work of the OPCW is the preparation to use designated laboratories where samples collected during an inspection could be sent for analysis. To be able to rely on the results provided by the designated laboratories, their performance must be rigorously tested. This is the purpose of the OPCW proficiency tests. In order to become a designated laboratory, the laboratory must have a national accreditation (ISO/IEC 17025 or equivalent). If FTIR is to be used in accredited testing, the laboratory must have amongst others written procedures for checking the performance of the instrumentation and for the analysis. The purpose of these checks is to guarantee high quality and reliability of the results.

The procedures can be adapted from standards or from the instrument manufacturer and they can be defined by the laboratory.

For example, ASTM has described a two-level procedure for checking the performance of an FTIR spectrometer ⁽⁷⁴⁾. The routine check of instrument performance, a so-called Zero-level test, includes the following tasks:

- measurement and comparison of the background against a previously measured background;
- measurement of a hundred per cent line (HPL); and
- measurement and comparison of a spectrum of a polystyrene sample against a previously measured spectrum.

The more extensive tests, so-called Level-one tests, include six tests, which facilitate the diagnostics of the instrument:

- energy spectrum test;
- HPL;
- stability test;
- signal-averaging test;
- polystyrene sample test; and
- photometric jitter test.

The performance of the GC must be tested regularly, for example, daily or after every 20th run, to ensure that the column retains no previously analyzed chemicals. When samples containing free acids are analyzed, for example, soil samples containing humic acids, the column should be tested before other types of samples. When the column performance degrades, the column should be changed or at least part of the column from the beginning should be replaced with a new piece of column. In order to lengthen the column's life, especially due to acidic injections, an uncoated deactivated precolumn can be used before the actual analytical column. There is a published Recommended Operating Procedure (ROP) for testing the performance of a GC system in the analysis of chemicals related to the CWC ⁽¹⁴⁾.

All the solvents used must be checked before the analysis, and during the analyses relevant solvent blanks must be used to test the cleanness of the system. When analyzing derivatized samples, it is not enough to use just the solvent, but the derivatizing chemicals should also be added to the solvent to ensure that there are no derivatizable chemicals in the system before the actual analysis.

If a blank is supplied for each sample, as often has been the case in international tests so far, they should be analyzed before the samples. It should be remembered that in realistic cases no blank samples are available or that the blank and the sample may have totally a different background, for example, water coming into a factory (blank) and wastewater (sample with possible chemicals).

There should also be some criteria for the quality of spectra. OPCW has used criteria for the reference spectra (see Section 3.3.3). These criteria can also be used for sample spectra where applicable.

6 COMPARISON WITH OTHER ANALYTICAL METHODS

In an analysis of a complicated sample, GC/FTIR alone cannot fulfill the identification criteria, just like any other analytical technique. Results from one technique can be correct but still for the final proof of the correctness, consistent results from other techniques are required, preferably from two spectrometric techniques.

Compared to GC/FTIR, GC/EI/MS gives comparable chromatograms, but is generally a more sensitive technique. The structural information provided by GC/FTIR and GC/EI/MS differs so that they complement each other well. For example, there are cases when EI/MS cannot differentiate between isomers but FTIR can, and cases in which FTIR cannot tell the exact size of an alkyl group but EI/MS can. These techniques also give overlapping structural information thus increasing the reliability of the identification. Commercial EI/MS spectral libraries are cheaper and larger than FTIR spectral libraries. Therefore, it is easier to routinely identify chemicals present in the samples by GC/EI/MS and disregard the uninteresting chemicals on the basis of the library match.

GC/CI/MS gives information on molecular mass. It can also give information on some substituents if CI/MS/MS is used. The molecular weight information becomes more important as the size of the molecule increases since FTIR cannot easily distinguish the sizes of longer alkyl groups.

Other mass spectral techniques that use LC and capillary electrophoresis (CE) as the sample introduction method make it possible to analyze chemicals that should otherwise be derivatized for GC analysis, and also those nonvolatile and nonderivatizable chemicals that cannot be analyzed at all with GC. Many of these chemicals could be analyzed with FTIR without GC separation, but in the environment, they may be in, for example, water or soil samples (which possibly have to be extracted with water). Water samples are difficult to analyze with FTIR since water is quite a poor solvent for FTIR due to very high molar absorptivity.

NMR is one of the most important tools in structural elucidation, but since the technique cannot be hyphenated with GC like MS and FTIR and its sensitivity is poorer, it cannot always identify chemicals from high background samples. The unchallenged capability of NMR to give information on connectivity and neighboring atoms makes it a very desirable method in the analysis of chemicals related to the CWC. NMR can, for instance, normally find out the type of the carbons (methyl, ethyl, propyl, or isopropyl) directly connected to phosphorus (^1H and ^{31}P spectra) and the presence of fluorine-phosphorus link (^1H , ^{19}F , and ^{31}P spectra). Different 2D-NMR experiments give additional information on connected atoms.

FTIR is a valuable addition to the instrumentation for a laboratory that is expanding its capabilities beyond just the basic necessities (GC and GC/MS). The effective and complementary combination of analytical techniques based on different principles, for example, like in IR, MS, and NMR, gives a solid base for any type of instrumental analysis. It is essential for the analysis related to the CWC that the analysis results are reliable and indisputable.

ABBREVIATIONS AND ACRONYMS

AMDIS	Automated Mass Spectral Deconvolution and Identification System
ASTM	American Society of Testing and Materials
ATR	Attenuated Total Reflectance
BSTFA	Bis(Trimethylsilyl)Trifluoroacetamide
CE	Capillary Electrophoresis
CWA	Chemical Warfare Agents
CWC	Chemical Weapons Convention
DLATGS	Deuterated L-Alanine Triglycine Sulfate
DMEP	Dimethyl Ethylphosphonate
DMIP	Dimethyl Isopropylphosphonate
DMMP	Dimethyl Methylphosphonate
DMPP	Dimethyl Propylphosphonate
DMT	Dimercaptotoluene
DRIFT	Diffuse Reflectance Infrared
FGC	Fourier Transform Chromatograms
FID	Flame-Ionization Detector
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC/CI/MS	Gas Chromatography/Chemical Ionization/Mass Spectrometry
GC/CI/MS/MS	Gas Chromatography/Chemical Ionization/Tandem Mass Spectrometry
GC/EI/HRMS	Gas Chromatography/Electron Ionization/High-resolution Mass Spectrometry
GC/EI/MS	Gas Chromatography/Electron Ionization/Mass Spectrometry

- | | | |
|---------|--|--|
| GC/FTIR | Gas Chromatography/Fourier Transform Infrared Spectroscopy | National Institute of Standards and Technology (NIST), Gaithersburg, MD, 2004, http://chemdata.nist.gov/ . |
| GC/MS | Gas Chromatography/Mass Spectrometry | 2. R. White, <i>Chromatography/Fourier Transform Infrared Spectroscopy and its Applications</i> , Marcel Dekker, New York, 1990. |
| GSC | Gram–Schmidt Chromatogram | 3. B. Stuart, <i>Modern Infrared Spectroscopy</i> , John Wiley & Sons, Ltd, Chichester, 1996. |
| HCI | Haz[ardous]Mat[erial] Chemical Identifier | 4. P.R. Griffiths and J.A. deHaseth, <i>Fourier Transform Infrared Spectroscopy</i> , John Wiley & Sons, Ltd, New York, 1986. |
| HPL | Hundred Per Cent Line | 5. L.C. Thomas <i>Interpretation of the Infrared Spectra of Organophosphorus Compounds</i> , Heyden & Sons Ltd., London, 1974. |
| HPLC | High-performance Liquid Chromatography | 6. ASTM Standard E 1252-94, <i>Standard Practice for General Techniques for Qualitative Infrared Analysis</i> , American Society for Testing and Material, Philadelphia, February 1995. |
| HQI | Hit Quality Index | 7. G. Dent, Preparation of samples for IR spectroscopy as KBr disks, <i>Internet J. Vib. Spect.</i> , 1 , 2–5 (1997). http://www.ijvs.com/volume1/edition1/section1.html#page2 . |
| IR | Infrared Spectroscopy | 8. W. Maddams, The background to sample preparation for infrared transmission measurements on solids, <i>Internet J. Vib. Spect.</i> , 1 , 6–11 (1997). http://www.ijvs.com/volume1/edition1/section1.html#page6 . |
| IRD | Infrared Detector | 9. <i>Jane's Nuclear, Biological and Chemical Defence</i> , http://jnbj.janes.com/ . |
| LC/FTIR | Liquid Chromatography/Fourier Transform Infrared Spectroscopy | 10. E.H. Braue, Jr. and M.G. Pannella, CIRCLE CELL FT-IR analysis of chemical warfare agents in aqueous solutions, <i>Appl. Spectrosc.</i> , 44 , 1513–1520 (1990). |
| MCT | Mercury–Cadmium–Telluride | 11. SensIR Technologies, Press Release January 28, 2003, <i>Advanced Technology Helps UN Weapons Inspectors Keep Pace – UN Team Relies on SensIR Technologies Travel IR HCI for Fast, Accurate Chemical Identification</i> . |
| MI | Matrix Isolation | 12. B. Erickson, Is there new life ahead for hyphenated IR? <i>Anal. Chem.</i> , 70 , 801A–805A (1998). |
| MS | Mass Spectrometry | 13. ASTM Standard E 1642-94, <i>Standard Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis</i> , American Society for Testing and Material, Philadelphia, February 1995. |
| MTBSTFA | <i>N</i> -Methyl- <i>N</i> -(<i>tert</i> -Butyldimethylsilyl)Trifluoroacetamide | 14. M. Rautio (Ed.), <i>Recommended Operating Procedures for Sampling and Analysis in the Verification of the Chemical Disarmament</i> , The Ministry for Foreign Affairs of Finland, Helsinki, 1994. |
| NIST | National Institute of Standards and Technology | |
| NMR | Nuclear Magnetic Resonance | |
| OCAD | OPCW Central Analytical Database | |
| OPCW | Organization for the Prohibition of the Chemical Weapons | |
| PAS | Photoacoustic Spectroscopy | |
| ROP | Recommended Operating Procedure | |
| SFC | Supercritical Fluid Chromatography | |
| TBDMS | <i>tert</i> -Butyldimethylsilyl | |
| TIC | Total Ion Chromatogram | |
| TMS | Trimethylsilyl | |
| UNMOVIC | United Nations Monitoring, Verification and Inspection Commission | |
| VERIFIN | Finnish Institute for Verification of the Chemical Weapons Convention | |

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CHAPTER 15

Capillary Electrophoresis in Analysis of Chemicals Related to the Chemical Weapons Convention

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1 INTRODUCTION

Since first developed in the early 1980s ⁽¹⁾, capillary electrophoresis (CE) has developed into a powerful analytical technique capable of separating compounds ranging from large biological molecules to small inorganic ions. The high efficiency and relatively short analysis times have made CE a promising alternative to conventional chromatographic techniques for numerous applications.

The ability to monitor compounds related to the Chemical Weapons Convention has become an increasingly important issue in recent years. Sensitive analytical techniques are required for identifying chemical weapons at suspected attack sites and for monitoring alleged production facilities. Most chemical warfare (CW) agents degrade after

relatively short exposure to the environment. For instance, organophosphate nerve agents degrade into alkylphosphonates and alkylphosphonic acids. Specifically, the nerve agent GB (Sarin) undergoes a rapid hydrolysis to form isopropyl methylphosphonate (IMPA), which then undergoes a much slower hydrolysis to form methylphosphonic acid (MPA). Therefore, it is generally these degradation products that are detected in the environment. Table 1 lists the organophosphate degradation products that have been analyzed by CE and sulfur-containing compounds related to the decomposition of sulfur mustard and other materials analyzed by CE can be found in Table 2.

As alkylphosphonates and alkylphosphonic acids are small ionic compounds, they are ideally suited for determination by CE. Sample preparation is minimal and derivatization is not required, as in

Chemical Weapons Convention Chemicals Analysis: Sample Collection, Preparation and Analytical Methods.

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Table 1. CWC-related organophosphorus compounds analyzed by capillary electrophoresis

Structure	R ₁	R ₂	Name	CAS ^a #	Reference
Alkylphosphonic acids					
$\begin{array}{c} \text{O} \\ // \\ \text{R}_1-\text{P}-\text{OH} \\ \\ \text{OH} \end{array}$	CH ₃		MPA	363067	9,12–23,30,31
	CH ₃ CH ₂		EPA	1782264	9,12,14,16,18,22,24
	CH ₃ CH ₂ CH ₂		PPA	4672-38-2	9,12,18,22,24
Alkylphosphonates					
$\begin{array}{c} \text{O} \\ // \\ \text{R}_2-\text{P}-\text{OR}_1 \\ \\ \text{OH} \end{array}$	CH ₃	CH ₃	MMPA	1066-53-1	9,15
	CH ₃ CH ₂	CH ₃	EMPA	1832-53-7	9,13–17,19,21–24,30,31
	(CH ₃) ₂ CH	CH ₃	IMPA	1832-54-8	9,13–17,19,21–24,30,31
	Cyclohexyl	CH ₃	CHMPA	1932-60-1	14–16,21,22,24
	(CH ₃) ₂ CHCH ₂	CH ₃	iBMPA	1604-38-2	16
	Pinacolyl	CH ₃	PMPA	616-52-4	9,13–17,19,21–24,30,31
	(CH ₃) ₂ CH(CH ₃)CH	CH ₃	DMPA	151299-67-1	14,15
	Cyclopentyl	CH ₃	CPMPA	73207-99-5	21,22
	2-Ethylhexyl	CH ₃	EHMPA	13688-82-9	16
	CH ₃ CH ₂	CH ₃ CH ₂	EEPA	7305-61-5	14,15
	(CH ₃) ₂ CH	CH ₃ CH ₂	iPEPA	170135-50-9	15,16
	(CH ₃) ₂ CH(CH ₃)CH	CH ₃ CH ₂	DEPA	195158-11-3	15
	Cyclohexyl	CH ₃ CH ₂	CEPA	170424-87-0	14,15
Methylphosphonothioic acids					
$\begin{array}{c} \text{O} \\ // \\ \text{CH}_3-\text{P}-\text{OR}_1 \\ \\ \text{SR}_2 \end{array}$	CH ₃ CH ₂	H	EMPSA	18005-40-8	21–25
	(CH ₃) ₂ CHCH ₂	H	IBMPA	20626-99-7	24,25
	H	(i-Pr) ₂ CH ₂ CH ₂ N	EA 2192	73207-98-4	24

^aChemical Abstracts Service.

the case of gas chromatography/mass spectrometry (GC/MS). While techniques such nuclear magnetic resonance (NMR) and mass spectrometry may be required for absolute identification of CW agent degradation products, CE can be employed for rapid screening of samples on site. The development of chip-based CE ⁽²⁾ has even created the possibility for handheld devices. Several governments worldwide have been attracted by this technology, and many fund active research programs for the development of portable devices based on capillary electrophoresis.

aspects of CE are discussed here to introduce CE and to review important fundamental issues for the analysis of chemical weapons convention (CWC) related compounds. These issues can affect the quality of CE separations, detection of target compounds, and method development. Specialized CE methods, such as capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachopheresis (CITP), and capillary electrochromatography (CEC) are beyond the scope of this article.



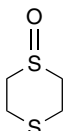
2.1 History

2 PRINCIPLES OF CAPILLARY ELECTROPHORESIS

Capillary electrophoresis has been the subject of extensive reviews and there are several books and publications describing the technique ^(3,4). Key

Electrophoresis refers to the separation of solutes based on their differential migration in an electric field. The velocity of charged solutes is proportional to the applied voltage. Thus, high voltage is theoretically desirable for fast and efficient separations. In practice, the use of high electric fields

Table 2. CWC-related degradation products of sulfur mustard, lewis, and other compounds analyzed by capillary electrophoresis

Structure	Name	CAS #	Reference
Sulfur mustard-related compounds			
$\text{HOCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{OH}$	TDG	111-48-8	24,25
$\text{HOCH}_2\text{CH}_2\text{S(O)CH}_2\text{CH}_2\text{OH}$	TDGO	3085-45-8	24,25
$\text{HOCH}_2\text{CH}_2\text{S(O)}_2\text{CH}_2\text{CH}_2\text{OH}$	TDGO ₂	2580-77-0	24
	1,4-Thioxane	15980-15-1	24,25
	1,4-Dithiane	505-29-3	24,25
	1,4-Dithiane-1-Oxide	19087-70-8	24
Lewisite-related compounds			
ClCHCHAs(O)	CVAOA	3088-37-7	24
Schedule 2 B Precursors			
$(\text{CH}_3\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{OH}$	DEAE	100-37-8	24
$[(\text{CH}_3)_2\text{CH}]_2\text{NCH}_2\text{CH}_2\text{OH}$	DIPAE	96-80-0	24
$[(\text{CH}_3)_2\text{CH}]_2\text{NCH}_2\text{CH}_2\text{SH}$	DIPAESA	1439975	24

is limited due to the unwanted peak broadening generated by Joule heating. As a result, early electrophoresis was performed in anticonvective media; for example, polyacrylamide gel, with low applied electric potentials. The analytes were also limited to macromolecules with low diffusion coefficients. Today, polyacrylamide gel electrophoresis (PAGE) has become a classical technique in biochemistry laboratories for separating macromolecules such as proteins, peptides, and nucleic acids.

Owing to the use of viscous gel media and low applied voltage, gel electrophoresis suffers from long analysis times and low efficiencies. Alternatively, the use of narrow bore tubes or capillaries was proposed as it provides good heat dissipation, allowing electrophoresis to be performed in solution instead of gel. It was soon realized that capillaries with smaller inner diameter (ID) provided the greatest heat dissipation due to a larger surface area to volume ratio ^(1,5). Today, CE is mostly performed in 10 to 100 μm ID (150 to 375 μm outer diameter) fused silica capillaries. This fragile capillary is protected by a polyimide outer coating, which

makes the capillary flexible and easy to handle. The high surface-to-volume ratio of the narrow capillaries allows for very efficient dissipation of Joule heating. In addition, the use of capillaries in electrophoresis allows separation of a wide variety of analytes ranging from small inorganic ions to large biomolecules.

2.2 Basic CE Operation

To perform a CE separation, the capillary is filled with the electrolyte by applying an external pressure to the inlet reservoir. Then the inlet reservoir is replaced with a sample reservoir, from which a small plug of sample is pushed into the capillary by either applying a small pressure or voltage. Typical injection volumes are in the order of 1 – 10 nL. Next, the inlet reservoir is put back in place, and separation begins upon the application of an electric field. Typical applied voltages are 100 to 1000 V/cm. Ions in the sample migrate with an electrophoretic mobility determined by their

charge and mass. Photometric detection is usually performed on capillary near the outlet reservoir. A window for optical detection is created by removing a section of the polyimide coating.

2.3 Electrophoretic Mobility

Separation by electrophoresis is based on the differences in the analytes' electrophoretic mobility. The electrophoretic mobility of an ion is proportional to the electric force that the ion experiences and inversely proportional to its frictional drag through the medium. Electrophoretic mobility can be expressed as

$$\mu_e = \frac{q}{6\pi\eta r} \quad (1)$$

where q is the net charge of the ion, η is the viscosity of the medium, and r is the radius of the ion. Therefore, electrophoretic separation occurs for solutes with different mobilities, or charge-to-size ratios.

2.4 Electroosmotic Flow (EOF)

In addition to the electromigration of analyte ions, electroosmotic flow (EOF) also occurs upon application of an electric field in CE. The inner walls of fused silica capillaries possess an intrinsic negative charge due to the presence of weakly acidic silanol groups ($-\text{SiOH}$). Cations in solution build up near the capillary surface to balance this charge, thus forming an electrical double layer. Upon the application of an electric field across the length of the capillary, the cations in the diffuse portion of the double layer migrate toward the negative electrode (cathode). Since these cations are hydrated, they induce a bulk flow of solution within the capillary toward the cathode that exhibits a flat flow profile. The magnitude of this EOF is generally described by the Smoluchowski equation:

$$\mu_{\text{eof}} = -\frac{\varepsilon\zeta}{\eta} \quad (2)$$

where ε and η are the dielectric constant and viscosity of the solvent, and ζ is the zeta potential. The zeta potential is slightly off the silica surface at

the plane of shear, and is a function of the deprotonation of the silanols, ion adsorption onto the surface, and the ionic strength of the buffer. Manipulation of the magnitude and/or the direction of the EOF can yield either rapid or high-resolution separations.

2.5 Modes of Operation

Capillary electrophoresis is a general term that is used to describe a number of different separation techniques. Capillary zone electrophoresis (CZE) is the classic technique and is therefore usually referred to as just CE. Other techniques include micellar electrokinetic chromatography (MEKC), capillary isoelectric focusing, and capillary isotachopheresis. CZE and MEKC are the predominant techniques and are those used herein, so only they will be discussed in detail here.

2.5.1 Capillary Zone Electrophoresis (CZE)

As described above, charged analytes in CZE experience both their electrophoretic mobility (μ_e) and also the electroosmotic flow mobility (μ_{eof}). The net or apparent mobility is given by

$$\mu_{\text{app}} = \mu_e + \mu_{\text{eof}} = \frac{L_d}{t_m E} \quad (3)$$

This apparent mobility can be easily calculated with the capillary length to the detector (L_d), the migration time of the analyte (t_m), and the electric field strength (E).

In general, most analytes separated by CE possess an electrophoretic mobility less than that of the EOF mobility (μ_{eof} is typically 5×10^{-4} to $8 \times 10^{-4} \text{ cm}^2/(\text{V s})$ at neutral pH). This allows for simultaneous determination of both positively and negatively charged species when the detector is placed at the cathodic (negative) end of the capillary. In this case, cations migrate in the same direction as the EOF (co-EOF) and anions migrate opposite to the direction of the EOF (counter-EOF). As long as anions have electrophoretic mobilities less than that of the EOF ($\mu_e < \mu_{\text{eof}}$), they are swept by the EOF toward the detector. Figure 1(a) shows a schematic of a CZE separation and Figure 1(b)

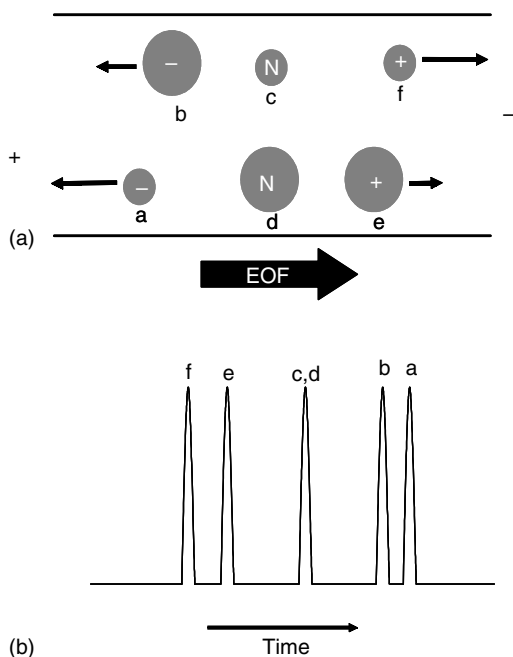


Figure 1. (a) Depiction of the movement of anions, cations, and neutral compounds under CZE conditions (b) and the corresponding theoretical electropherogram

displays the corresponding theoretical 'electropherogram'. Note that CZE is incapable of separating neutral compounds.

In the case of high-mobility analytes such as inorganic anions, μ_e values are comparable or even greater than that of μ_{eo} . For instance, common anions such as chloride, bromide, and sulfate have electrophoretic mobilities of 7.92, 8.09, and $8.29 \times 10^{-4} \text{ cm}^2/(\text{V s})$, respectively. Thus, these anions migrate faster than the EOF but in the opposite direction. As a consequence, they would not be detected in the normal configuration described above (detector at cathode). Rather one would need to inject at the cathode and place the detector at the anode to detect these ions.

The major source of band-broadening in CZE is longitudinal diffusion. Longitudinal diffusion refers to the axial diffusive spreading of the solute from the solute zone into the bulk solution as it travels down the capillary. The variance in peak width contributed by longitudinal diffusion is given by

$$\sigma_{\text{diff}}^2 = 2 D t_m \quad (4)$$

where D is the diffusion coefficient of the solute and t_m is the migration time. Hence, longitudinal diffusion is proportional to the time the solute spends in the capillary, and longitudinal diffusion is more severe for solutes with large diffusion coefficients. Therefore, it is desirable to use large applied voltages in CE (E in Equation (3)) in order to speed up the solutes and shorten the analysis time for minimized longitudinal diffusion. However, if excessive current is generated in the capillary (i.e. using high-conductivity buffers or large ID capillaries), Joule heating can limit the maximum electric field strength used. Joule heating creates a radial temperature gradient, with higher temperatures at the center of the capillary than at the walls. This temperature gradient gives rise to viscosity differences in the buffer and leads to band-broadening, as analyte molecules in the center of the capillary travel faster than those near the walls.

Neglecting all other sources of band-broadening, the maximum theoretical efficiency (N_{max}) due to longitudinal diffusion alone is

$$N_{\text{max}} = \frac{L_d^2}{2 D t_m} \quad (5)$$

where L_d is the capillary length measured from the inlet to the detector. The high efficiency achievable by CE (typically $> 100\,000$ plates) also enhances its resolving power. The resolution (R) of two components achievable in CZE is expressed by

$$R = \frac{1}{4} \sqrt{N} \frac{\Delta\mu}{\bar{\mu}_{\text{app}}} \quad (6)$$

where N is the efficiency, $\Delta\mu$ is the mobility difference between the ions and $\bar{\mu}_{\text{app}}$ is the mean apparent mobility of the ions.

2.5.2 Micellar Electrokinetic Chromatography (MEKC)

First reported in 1984 by Terabe and coworkers, MEKC is a hybrid of electrophoresis and chromatography⁽⁶⁾. Its main strength is its ability to separate both charged and neutral compounds.

Separation by MEKC is based on the differential partitioning of solutes into a 'pseudo'-stationary phase. In general, the pseudo-stationary phase is

formed simply by the addition of surfactants to the background electrolyte. Above their critical micelle concentration (CMC), surfactant molecules aggregate in such a fashion as to minimize the contact of their hydrophobic tails with water. Spherical aggregates are the most common result, with the hydrophobic tails oriented toward the center and the hydrophilic head groups on the exterior. A schematic of a micelle is shown in Figure 2(a). The anionic surfactant sodium dodecyl sulfate is the classic surfactant employed for MEKC and has a CMC of ~ 8 mM and an aggregation number of ~ 60 .

A schematic of the separation mechanism of MEKC is shown in Figure 2(a) and Figure 2(b) displays the corresponding theoretical chromatogram. Briefly, the negatively charged sodium dodecylsulfate (SDS) micelles migrate toward the anode (positive), which is in the opposite direction of the EOF. At neutral or basic pH, the EOF velocity will be fast enough such that the net movement of the micelles will be toward the cathode (negative). A completely unretained (hydrophilic; no interaction

with micelles) neutral analyte will migrate with the EOF and will elute at t_0 , as shown in Figure 2(b). Conversely, a neutral analyte that is completely retained by the micelles will elute at t_{mc} , which corresponds to the time it takes a micelle to travel from the capillary inlet to the detector. Any neutral analytes with any degree of retention falling between these two extremes must then elute between t_0 and t_{mc} , with more highly retained compound eluting after less retained compounds (Figure 2(b)). The length of this time window ($t_{mc} - t_0$) is significant in MEKC in that it dictates the peak capacity. Thus, it is advantageous to use micelles having high mobility in the direction opposite of the EOF.

Retention of neutral solutes in MEKC is essentially chromatographic and can be described using modified chromatographic relationships. The capacity factor, \tilde{k}' , is given by

$$\tilde{k}' = \frac{(t_r - t_0)}{t_0 \left(1 - \frac{t_r}{t_m}\right)} \quad (7)$$

where t_r is the retention time of the solute, t_0 is the retention time of an unretained solute (dead time), and t_{mc} is the micelle elution time (100% retained compound). This equation is modified from the standard chromatographic form to account for the movement of the pseudo-stationary phase. Retention is most easily adjusted in MEKC by varying the surfactant concentration. Generally, the capacity factor increases linearly with surfactant concentration. However, the range of ionic surfactant concentrations is limited, since high concentrations can produce excessive current and thus lead to Joule heating in the capillary (temperature gradient). The typical concentration range for SDS is 20 – 100 mM.

Resolution of two components in MEKC is given by

$$R = \left(\frac{\sqrt{N}}{4}\right) \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k'_2}{k'_2 + 1}\right) \left(\frac{1 - \left(\frac{t_0}{t_m}\right)}{1 - \left(\frac{t_0}{t_m}\right)k'_1}\right) \quad (8)$$

where N is the number of theoretical plate (typically 100 000–200 000) and α is the separation factor (k'_2/k'_1). By far the most powerful term in this

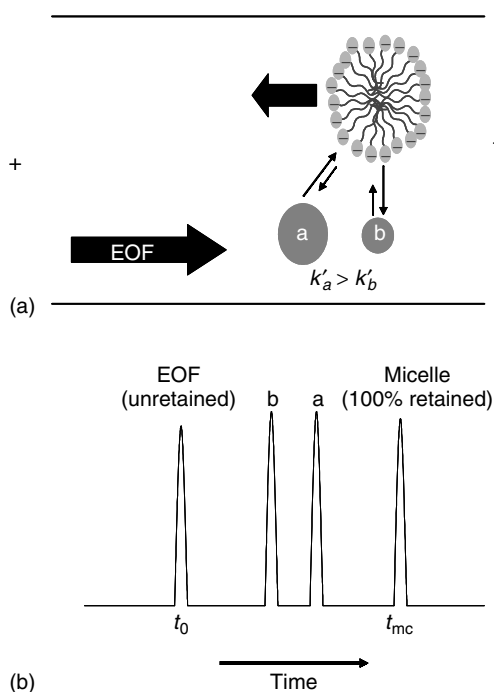


Figure 2. (a) Schematic of the separation mechanism of MEKC (b) and the corresponding theoretical electrochromatogram

equation for increasing resolution is selectivity term ($\alpha - 1/\alpha$). Altering the surfactant type is an effective way to alter selectivity. Zwitterionic surfactants such as CHAPS and bile salts such as cholate have both been used effectively. Buffer additives such as cyclodextrins have also been used to alter selectivity in MEKC. Cyclodextrins introduce a shape or orientation component into the overall retention mechanism and can be used in conjunction with surfactants to fine-tune separations. Generally speaking, the resolving power of MEKC is such that separation can be achieved for a pair of analytes with α as low as 1.02.

2.6 Detection

Owing to the very small dimensions of the capillaries used in CE, adapting conventional HPLC-style detectors to CE has been problematic. Although great improvements in sensitivity have been achieved over the last 20 years, the development of detection schemes for CE remains an active area of research.

On-column UV absorbance detection is by far the most common method of detection in CE today. Many compounds of interest absorb light to some extent in the UV region without any chemical modification. Detector components are fairly robust and inexpensive, and little operator skill is required. For these reasons, most commercial CE instruments are equipped with a standard UV absorbance detector. However, as absorbance signals are directly proportional to the optical pathlength (Beer's Law), the 10–100 μm internal diameter of capillaries used in CE yield rather disappointing detection limits in the range of 10^{-5} – 10^{-7} M ⁽⁷⁾.

Arguably, fluorescence has emerged as the most sensitive detection technique in CE. Because of the difficulty with focusing conventional light sources such as xenon and mercury lamps, lasers are generally employed for excitation. Laser-induced fluorescence (LIF) can routinely achieve limits of detection in the 10^{-9} – 10^{-12} M range ⁽⁷⁾ and has even attained single-molecule detection ⁽⁸⁾. However, fluorescence detection generally involves derivatization of analytes with a fluorescent probe.

Electrochemical techniques such as conductivity, amperometry, and potentiometry have all been

applied successfully to detection in CE with detection limits in the range of 10^{-5} to 10^{-8} M ⁽⁷⁾. However, practical problems with the precise placement of microelectrodes and the grounding of the electrophoretic current prior to the point of detection have restricted their development into standard methodology. Electrospray ionization/mass spectrometry (ESI/MS) has also been implemented successfully as a detection system in CE separations and commercial systems have become available.

3 CE ANALYSIS OF CWC-RELATED COMPOUNDS

3.1 CE Analysis of Organophosphate Degradation Products

Alkylphosphonates and alkylphosphonic acids are relatively strong acids with $\text{p}K_a$ values ranging from roughly 1.7 to 2.4 and are therefore negatively charged under most CE buffer conditions. Therefore, the analytes will migrate in the opposite direction to the EOF (counter-EOF). However, the magnitude of the EOF under most conditions is sufficient to sweep all alkylphosphonates and alkylphosphonic acids to the detector. Under these conditions, low mobility compounds such as cyclohexylethylphosphonate (CHEPA) will reach the detector before higher-mobility analytes such as methylphosphonic acid (MPA), as seen in Figure 3. In cases where more rapid separations are desired, buffer additives can be employed to suppress or even reverse the direction of the EOF. Under either of these conditions, the voltage polarity is reversed and analytes will migrate to the detector in order of increasing μ_e , as shown in Figure 4.

While the separation of alkylphosphonates and alkylphosphonic acids by CE is rather straightforward, sensitive detection remains a challenging task. Since these analytes do not contain a chromophore or a fluorophore, and are not easily derivatized, standard optical detection techniques such as absorbance or fluorescence cannot be employed. Representative methods for the determination of organophosphate degradation products are summarized below and grouped by the mode of detection employed. Experimental conditions for some of these methods are summarized in Table 3.

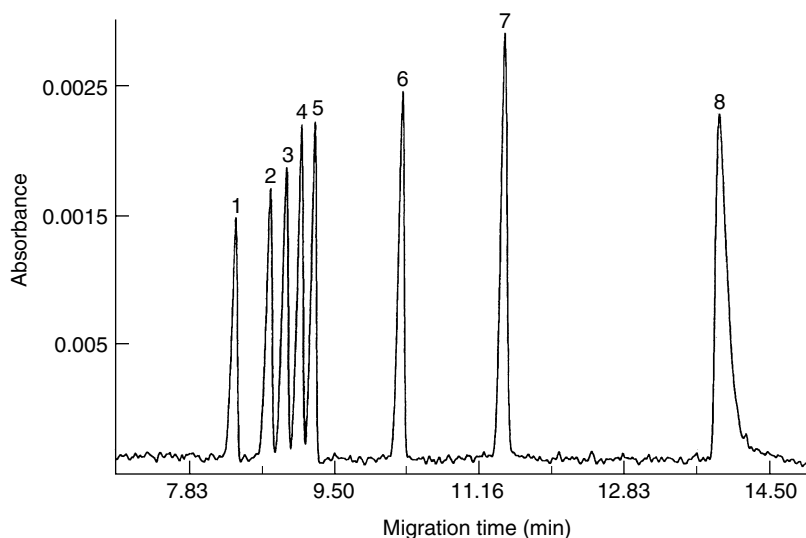


Figure 3. CZE electropherogram of a standard mixture of eight alkylphosphonates (solute concentration 20 mg L^{-1}): CEPA (1), PMPA (2), CMPA (3), DMPA (4), EEPA (5), IMPA (6), EMPA (7), and MPA (8). CZE conditions: 67 cm L_t (60 cm L_d) $\times 50 \mu\text{m ID}$; $V = 30 \text{ kV}$; Buffer: 5 mM sorbic acid, 0.1 mM decamethonium bromide, $\text{pH } 6$; indirect UV detection at 254 nm . (Reprinted from J.-P. Mercier, P. Morin, M. Dreux, and A. Tambute, *J. Chromatogr. A*, **741**, 279–285 (1996), reproduced with permission from Elsevier Science)

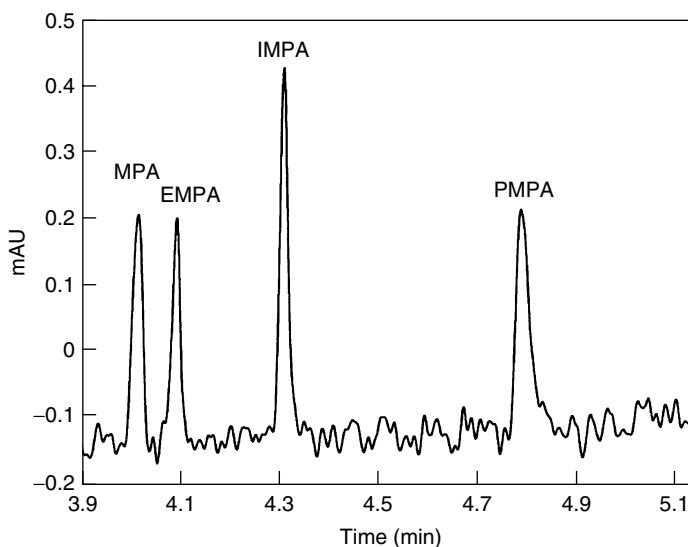


Figure 4. CZE electropherogram of a standard mixture of 4 alkylphosphonates (solute concentration 1 mg L^{-1}) CZE conditions: 50 cm L_t (40 cm L_d) $\times 50 \mu\text{m ID}$; $V = -20 \text{ kV}$; Buffer: 10 mM glutamic acid, 1 mM phenylphosphonic acid, 1 mM CAS-U, $\text{pH } 3.22$; indirect UV detection at 214 nm . (Reprinted from J.E. Melanson, B.L.Y. Wong, C.A. Boulet, and C.A. Lucy, *J. Chromatogr. A*, **920**, 359–365 (2001), reproduced with permission from Elsevier Science)

Table 3. Representative CE methods for the analysis of CW degradation products

Class	Mode	Buffer	pH	Detection (λ , nm)	E (Vcm ⁻¹)	Reference
Alkylphosphonates	CZE	200 mM borate 10 mM phenylphosphonic acid	6	Indirect UV (254)	465	12
Alkylphosphonates	CZE	5 mM sorbic acid 0.1 mM decamethonium bromide	6	Indirect UV (254)	448	14
Alkylphosphonates	CZE	200 mM boric acid 10 mM phenylphosphonic acid 0.03 % wt % Triton X 0.35 % DDAOH	4	Indirect UV (210)	-536	30
Alkylphosphonates	CZE	20 mM ammonium acetate	9	ESI/MS	375	23
Methylphosphonothioates	CZE	250 mM borate	7	Direct UV (200)	470	24
S-mustard-related compounds	MEKC	10 mM borate 100 mM SDS	9	Direct UV (200)	440	25

3.1.1 Direct UV Detection

Direct UV detection of alkylphosphonic acids through the formation of sodium borate complexes has been reported⁽⁹⁾. While native alkylphosphonic acids exhibit negligible absorbance above 200 nm, borate complexation shifted their absorption maxima such that they were readily detected at either 214 nm or 254 nm. The buffer system consisted of 10 mM sodium tetraborate with complexation occurring in solution at pH 7 and higher. The reported detection limits on the order of nanograms. The UV, IR, NMR, and mass spectra of various borate/phosphonic acid esters were also reported.

3.1.2 Indirect UV Detection

When compounds are not optically active and are not easily derivatized, indirect detection is sometimes the best alternative. In indirect detection, an absorbing or fluorescing probe is added to the buffer. Displacement of the probe by the analyte produces a decrease in signal^(10,11). Because of its universality and simplicity, indirect UV absorbance detection has been the predominant detection scheme for phosphonic acid analyses by CE⁽¹²⁻¹⁷⁾.

In indirect detection, the theoretical limit of detection (LOD) is given by:

$$C_{\text{LOD}} = \frac{C_p}{T_R \times DR} \quad (9)$$

where C_p is the concentration of the probe, T_R is the transfer ratio (the number of probe molecules

displaced by 1 analyte molecule), and DR is the dynamic reserve (ratio of the background absorbance to the noise). Selection of an appropriate probe is critical for achieving good efficiency and sensitivity in indirect detection. Firstly, the probe should be highly absorbing at the wavelength employed such that C_p can be kept to a minimum while still generating a significant background absorbance. Further, since the mechanism of indirect detection is based on charge displacement, the probe should not be more highly charged than the analytes to maximize T_R . Finally, the probe should have an electrophoretic mobility similar to that of the analytes to minimize electrodispersion effects (broad, asymmetric peaks).

Capillary zone electrophoresis with indirect UV detection at 254 nm was first used for the detection of a series of alkylphosphonic acids⁽¹²⁾. Four probes were investigated including benzoic acid, phthalic acid, sorbic acid, and phenylphosphonic acid. Phenylphosphonic acid, which has a mobility close to that of the analyzed compounds, was shown to be the most suitable probe. The influence of several parameters, such as the concentration of the probe and the borate buffer on both sensitivity and efficiency, was investigated. An increase in the borate concentration produced an improvement of the signal-to-noise ratio, while the sensitivity decreased with increasing concentration of the phenylphosphonic acid. A pH 6, 200 mM sodium borate buffer containing 10 mM phenylphosphonic acid was selected and the separation of MPA, US Environmental Protection Agency (EPA), polarization propagator approximation (PPA), and bisphenol A (BPA) was demonstrated. The reproducibility of

the μ_e for MPA was 1.65 % relative standard deviation (RSD) and the limit of detection was less than 10 μM (0.21 pmol injected).

Sorbic acid has also been shown to be a suitable probe for the analysis of the alkylphosphonic acids and their monoester derivatives⁽¹⁴⁾. A run buffer consisting of 5 mM sorbic acid and 0.1 mM decamethonium bromide, adjusted to pH 6, was used to separate a mixture of eight alkyl alkylphosphonates. The use of the EOF modifier decamethonium bromide reduced the EOF at pH 6 to enhance the resolution and gave complete separation of the alkylphosphonic acids and their monoester derivatives (e.g. MPA and IMPA) in less than 15 minutes, as shown in Figure 3.

In cases where fewer analytes need to be resolved, buffer additives can be employed to expedite the separation. The zwitterionic surfactant coco (amido-propyl) ammoniumdimethylsulfobetaine (Rewoteric AM CAS U) has been employed for complete EOF suppression in reversed polarity mode⁽¹⁷⁾. Separation of MPA, ethyl methylphosphonic acid (EMPA), IMPA, and PMPA was achieved in less than 5 min, as shown in Figure 4. The phenylphosphonic acid concentration was lowered to 1 mM from the 10 mM used in previous literature studies, resulting in greater sensitivity. Detection limits of 2 μM were achieved with hydrodynamic injection and up to 100-fold lower using electrokinetic injection. Glutamic acid was used as a buffering agent at its isoelectric point (pH 3.22). In its zwitterionic form, glutamic acid does not act as a competing co-anion in the system, thus providing good buffering capacity while maintaining high sensitivity. The increased buffering capacity of this system over previous methods led to migration time reproducibility RSD values of 0.18 to 0.22 %⁽¹⁷⁾.

Reversal of the direction of the EOF using the cationic surfactant didodecyldimethylammonium hydroxide (DDAOH) in reversed polarity mode has also been reported⁽¹⁶⁾. The bromide form of this surfactant (DDAB (didodecyldimethylammonium bromide)) was converted to the hydroxide form to eliminate an undesirable system peak caused by bromide. Using 10 mM phenylphosphonic acid as the probe and a buffer consisting of 200 mM borate, 0.35 mM DDAOH, and 0.03 % Triton X-100 at pH 4.0, the separation of 8 alkylphosphonates was achieved in less than 3 min. Limits of detection were in the 100 $\mu\text{g/L}$ range.

3.1.3 Direct Fluorescence Detection

The alkylphosphonic acids, MPA, EPA, and PPA, have been determined by direct fluorescence after derivatization with 4-(9-anthroxyloxy)phenacyl bromide (panacyl bromide)⁽¹⁸⁾. The 325-nm line of the HeCd laser was used for excitation. The neutral derivatives required MEKC for the separation and 50 mM sodium cholate was used as the pseudo-stationary phase. Separation was achieved in 33 min and limits of detection ranged from 0.13–0.14 μM (12–17 ppb).

3.1.4 Indirect Fluorescence Detection

Similar to indirect absorbance detection, indirect fluorescence has been employed to detect MPA, EMPA, IMPA, and PMPA, using tetrakis(4-sulfophenyl)porphine (TSPP) as the indirect probe⁽¹⁹⁾. A violet diode laser operating at 415 nm was used for excitation. Using an electrolyte composed of 50 μM TSPP and 5 mM [Bios(2-hydroxyethyl)-amino]tris-(hydroxymethyl)methane (Bistris) at pH 7.2 under normal polarity, baseline separation was achieved in less than 2 min. A limit of detection of 0.1 μM (9 ppb) for MPA was achieved.

3.1.5 Flame Photometric Detection

The separation performance and sensitivity of a capillary electrophoresis system coupled to a phosphorous-specific flame photometric detector (FPD) has been reported for the detection of alkylphosphonic acids^(20–22). The liquid junction used to decouple the electric field from the FPD showed a negligible influence on the performance of the system as compared with online UV detection. The use of an on-column sample stacking preconcentration technique allowed for injection of 900 nL. With the large injection, the detection limits for the alkylphosphonic acids in water were 0.1–0.5 $\mu\text{g mL}^{-1}$.

3.1.6 Mass Spectrometric Detection

Mass spectrometry is the only technique to date, which has been coupled with CE to provide the structural information required for unambiguous

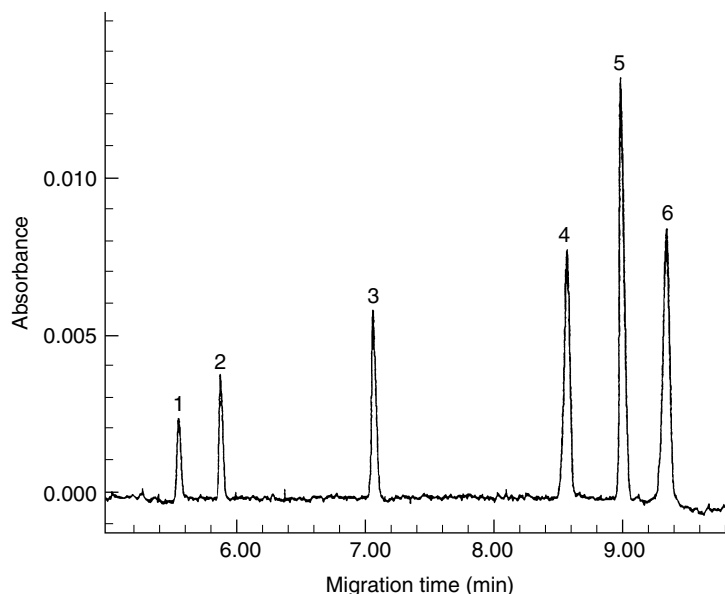


Figure 5. MEKC electropherogram of six sulfur-containing compounds related to the degradation of mustard and *O*-alkyl alkylphosphonothioates (solute concentration 50 mg L^{-1}): TDGO (1), TDG (2), Thioxane (3), IBMPSA (4), Dithiane (5), and EMPSA (6). MEKC conditions: 57 cm L_t (50 cm L_d) \times $75 \text{ }\mu\text{m ID}$; $V = 25 \text{ kV}$; Buffer: 10 mM borate, 100 mM SDS, $\text{pH } 9$; direct UV detection at 200 nm . (Reprinted from R.L. Cheicante, J.R. Stuff, and H.D. Durst, *J. Chromatogr., A*, **711**, 347–352 (1995), reproduced with permission from Elsevier Science)

identification of CW degradation products. Capillary zone electrophoresis/ion spray/mass spectrometry (CZE/IS/MS) combines the high separation efficiency of CE and the high sensitivity of mass spectrometry (MS) for the analysis of these polar degradation products. CZE/IS/MS, in the negative-ion mode, was applied to the identification of five organophosphonic acids that are the primary hydrolysis products of nerve agents⁽²³⁾. The spectra of EMPA, IMPA, and PMPA exhibited very abundant $(\text{M}-\text{H})^-$ ions of m/z 123, 137, and 179 respectively. The fragmentation was minimal and common fragmentation ions at m/z 95 and 79 were observed because of the loss of the alkyl and alkoxy groups respectively. LODs in the range $10\text{--}30 \text{ pg}$ ($5 \text{ ng }\mu\text{L}^{-1}$) were achieved using selected ion monitoring.

3.2 Sulfur-containing Compounds

The sulfur-containing, neutral degradation products of mustard and related vesicants can be analyzed by direct UV detection using MEKC. MEKC has

been used for the analysis of thiodiglycol (TDG), 2,2'-sulfinyldiethanol (TDGO), 1,4-dithiane, 1,4-thioxane, *O*-isobutyl methylphosphonothioic acid (IBMPSA) and *O*-ethyl methylphosphonothioic acid (EMPSA). These were separated in under 10 minutes with a run buffer of 10 mM borate and 100 mM SDS at $\text{pH } 9$ ^(24,25), as shown in Figure 5. Detection limits range from 1 to $10 \text{ }\mu\text{g mL}^{-1}$.

3.3 Toxins

3.3.1 Saxitoxin

Saxitoxin (STX) is a potent neurotoxin that can cause paralytic shellfish poisoning (PSP). Produced by certain strains of dinoflagellates, saxitoxin leads to the contamination of commercial shellfish and cause severe outbreaks of seafood poisoning. The public health problems caused by these outbreaks have led to significant interest in the development of analytical methods for the analysis of saxitoxin in environmental and biological samples. Saxitoxin is also one of a series of several closely related

compounds, so unambiguous identification of the target compound is a significant analytical requirement for its analysis.

Saxitoxin has been labeled with fluorescamine, *o*-phthalaldehyde (OPA) and dansyl chloride and detection limits as low as 0.1 attomole were reported for the OPA derivative of saxitoxin⁽²⁶⁾. Labeling, separation, and analysis of saxitoxin was best accomplished using fluorescamine, which produces ionic derivatives that can be separated from other fluorescently labeled marine toxins, such as tetrodotoxin and microcystin. However, the precolumn labeling methods required μM concentrations of analyte, limiting the utility of the technique for trace analysis.

CE analysis with direct UV absorbance detection at 200 nm has been described for the separation and detection of underivatized toxins, including saxitoxin, associated with paralytic shellfish poisoning⁽²⁷⁾. Confirmation of the electrophoretic peaks was made by CE/ESI/MS. Saxitoxin and neosaxitoxin (NEO) were separated using a 20 mM sodium citrate buffer at pH 2.1 yielding a mass LOD of 15 pg ($5 \mu\text{M}$) for saxitoxin.

The application of on-column sample preconcentration CITP and a discontinuous buffer system prior to CE separation has been investigated for the analysis of PSP toxins⁽²⁸⁾. Using a linear polyacrylamide-coated capillary to eliminate EOF during the CITP step, 35 mM morpholine (pH 5) as the leading electrolyte and 10 mM formic acid as the terminating electrolyte, the improvement of the concentration limit of detection (CLOD) is at least two orders of magnitude over that obtainable using the conventional CZE format. Analyses performed using selected ion monitoring ESI/MS provided LODs of 16 nM for saxitoxin. This preconcentration technique was compatible with ESI/MS and permitted the analysis of biological extracts containing PSP toxin levels as low as 100 nM.

3.3.2 Ricin

Ricin is a heterodimeric glycoprotein (MW = 66 000) produced by the castor bean plant *Ricinus communis* with the two protein subunits linked by a single disulfide bond. Conditions for the analysis of ricin with CE, using uncoated and coated capillaries as well the influence of buffer systems (run buffer

composition, pH, ionic strength, and additives) have been investigated⁽²⁹⁾. Uncoated columns used with either zwitterionic salts or putrescine gave the best results. Multiple peaks were resolved with these conditions. Analysis using a bare fused silica column with a 30 mM sodium borate run buffer gave a single, broad tailing peak, which could be due to protein-column interactions, additional components, or both. The use of 30 mM putrescine (1,4-diaminobutane) in 20 mM K_2SO_4 buffer, pH 7, gave better resolution and peak shape. Multiple peaks were resolved, indicating possible impurities or microheterogeneity in the ricin sample.

4 ENVIRONMENTAL ANALYSIS

The development of CE analytical methods for CW-related compounds has been directed primarily toward the analysis of these compounds in environmental matrices such as soil and water. These methods have been used in the analysis of samples distributed as part of the interlaboratory comparisons exercises, the so-called *round-robin exercises* coordinated by Finland, and more recently, the OPCW Laboratory Proficiency Testing.

In a recent series of papers, Nassar *et al.* demonstrated the utility of indirect UV-absorbance detection for environmental samples. In their first study, samples were collected from an area known to have been exposed to nerve agents and subsequently cleaned up⁽¹⁶⁾. Using the same buffer system as described above (Section 3.1.2), MPA, IMPA, and PMPA were detected in soil and surface wipe leachates. Interference from common anions was minimal, likely owing to the acidic buffer conditions employed (pH \sim 4), which can keep weak acids such as carboxylic acids and carbonic acid primarily in their protonated form. Further, interference from fluoride may have been reduced as it is known to adsorb to the capillary surface at low pH.

A validated method for IMPA and PMPA in reaction masses was also reported⁽³⁰⁾. After neutralization of GB (sarin) and GD (Soman) with aqueous monoethanolamine, IMPA and PMPA were determined through spiking. Linear calibration was achieved for both analytes over a range of 0.5–100 $\mu\text{g/ml}$. Intraday precision based on peak area ranged from

0.7 to 2.9% RSD for IMPA and PMPA over a range of concentrations. Migration time intraassay precision was less than 4.0% RSD for IMPA and 5.0% RSD for PMPA. Recovery of IMPA and PMPA in the spiked reaction masses was greater than 86%.

Finally, the same authors reported the incorporation of electrokinetic injection into their method⁽³¹⁾. Little interference was observed in the separation of MPA, EMPA, IMPA, and PMPA in seven different environmental matrices. Limits of detection were reduced to 1–2 µg/L for water samples, which represented an improvement of two orders of magnitude over their previous work using pressure injection. However, a sample cleanup step was necessary to reduce the conductivity of the sample to be compatible with electrokinetic injection. The samples were passed through three pretreatment cartridges arranged in series to remove sulfate, chloride, and cations, respectively.

ABBREVIATIONS AND ACRONYMS

BPA	Bisphenol A
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CGE	Capillary Gel Electrophoresis
CHEPA	Cyclohexylethylphosphonate
CIEF	Capillary Isoelectric Focusing
CITP	Capillary Isotachopheresis
CLOD	Concentration Limit of Detection
CMC	Critical Micelle Concentration
CW	Chemical Warfare
CWC	Chemical Weapons Convention
CZE	Capillary Zone Electrophoresis
CZE/IS/MS	Capillary Zone Electrophoresis/Ionspray/Mass Spectrometry
DDAOH	Didodecyltrimethylammonium Hydroxide
EMPA	Ethyl Methylphosphonic Acid
EMPSA	<i>O</i> -Ethyl Methylphosphonothioic Acid
EOF	Electroosmotic Flow
EPA	US Environmental Protection Agency
ESI/MS	Electrospray Ionization/mass Spectrometry

FPD	Flame Photometric Detector
GB	Sarin
GC/MS	Gas Chromatography/Mass Spectrometry
GD	Soman
IBMPA	<i>O</i> -Isobutyl Methylphosphonothioic Acid
ID	Inner Diameter
IMPA	Isopropyl Methylphosphonate
LIF	Laser-induced Fluorescence
LOD	Limit of Detection
MEKC	Micellar Electrokinetic Chromatography
MPA	Methylphosphonic Acid
MS	Mass Spectrometry
NEO	Neosaxitoxin
NMR	Nuclear Magnetic Resonance
OPA	<i>o</i> -Phthalaldehyde
PAGE	Polyacrylamide Gel Electrophoresis
PPA	Polarization Propagator Approximation
PSP	Paralytic Shellfish Poisoning
RSD	Relative Standard Deviation
SDS	Sodium Dodecylsulfate
STX	Saxitoxin
TDG	Thiodiglycol
TSPP	Tetrakis(4-sulfohenyl)Porphine

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CHAPTER 16

Methods for the Retrospective Detection of Exposure to Toxic Scheduled Chemicals. Part A: Analysis of Free Metabolites

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1 INTRODUCTION

1.1 General

In cases, or allegations of use of chemical warfare (CW) agents, environmental and biomedical samples may be collected for forensic analysis. Unequivocal

identification of agents, degradation products, metabolites, or covalently bound residues provide key supporting evidence of noncompliance with the Chemical Weapons Convention (CWC). The increasing terrorist threat of using CW agents has further stimulated the requirement for forensic methods of analysis. The Organization for the Prohibition of Chemical Weapons (OPCW) has established a

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network of designated laboratories that are expert in the analysis of environmental samples. Biomedical samples offer a greater challenge because of the low concentrations of analytes likely to be encountered, the greater complexity of biological matrices, and the initial requirement to identify suitable biological indicators of poisoning ⁽¹⁾. In addition to forensic applications, biomedical sample analysis may be used for diagnostic purposes to ensure that casualties receive appropriate therapy, and for monitoring exposure to CW agents, for example, in workers engaged in demilitarization activities.

Most CW agents are reactive electrophiles. They react with biological nucleophiles, such as water or glutathione, through chemical or enzyme-catalyzed reactions to give free metabolites that are excreted in urine. Other metabolic reactions, such as oxidation and *S*-methylation, also occur. In addition to free metabolites, covalent adducts are formed with nucleophilic sites on macromolecules. These may remain in the blood and tissues for much longer, depending on their turnover within the body. Both free metabolites and covalent adducts provide unequivocal biological indicators of exposure to CW agents. This chapter will review the metabolism of CW agents and methods for the analysis of free metabolites in biological fluids. The following chapter, Part B, will review covalent adducts formed with proteins and DNA.

1.2 Urinary Metabolites

Metabolites excreted in urine offer one of the simplest means of confirming that a casualty has been exposed to a CW agent. Urine has several advantages over other biomedical samples. The major fraction of an absorbed dose of a CW agent is likely to be eliminated as urinary metabolites, a much lesser amount being excreted in the feces. Urine is a simpler matrix than blood or tissue, and does not require invasive collection. It is generally free from proteins and lipids, and is therefore more easily extracted. It is however a very variable matrix in terms of pH (normal range pH 5.5–7) and concentration of inorganic and organic constituents. Conversely, collection of blood and tissue samples (other than hair) is invasive and requires medically competent personnel, but the composition of blood is more consistent than urine. The major disadvantage

of urinary metabolites as biological markers is that up to ~90 % of the total amount excreted may be eliminated in the first 48–72 h following an exposure. Figure 1 shows a typical excretion profile for urinary metabolites following systemic administration, a pattern that is likely to be followed after an acute inhalation exposure. It is characterized by a rapid rise to a maximum excretion rate, usually within a few hours, with up to 80–90 % being excreted within the first 2–3 days. Thereafter, a slower elimination phase is usually observed, which may arise from agents repartitioning out of fatty tissues or the slow release of metabolites from covalent adducts of the agent with macromolecules such as protein and DNA. Elimination of metabolites following cutaneous exposure is expected to follow a similar pattern, but the curve may be somewhat flatter resulting from the slower absorption by this route. It is clear from Figure 1 that a much more sensitive analytical technique is required to detect metabolites in urine samples collected several days following an exposure, compared to samples collected within the first 48 h. This is well illustrated by comparing samples collected from the Iraq–Iran conflict, with samples collected from the terrorist attack with sarin in the Tokyo subway (see Sections 2.3 and 5.3).

1.3 Blood Metabolites

In addition to urinary metabolites, free metabolites may be detected in blood, provided sampling

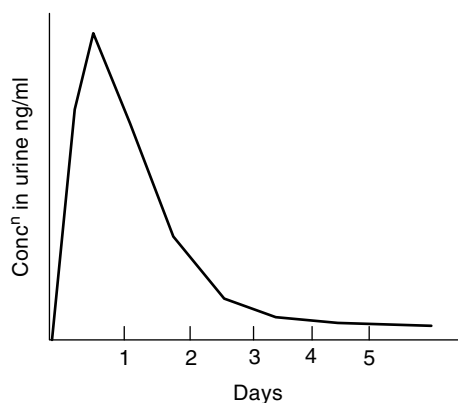


Figure 1. Typical urinary excretion profile of a CW agent metabolite

is undertaken relatively soon after the exposure. Examples are provided in Section 5.3 where blood metabolites were detected in casualties of a terrorist attack in Japan. A disadvantage of blood is that it is a more difficult matrix to work with compared to urine. It is a buffered fluid containing suspended cells, proteins, fats, and salts. Fresh blood is easily separated into red cells and plasma (or serum if coagulated), the latter being a much easier matrix. If handled inappropriately (e.g. heating, freezing, agitation, dilution with water), red cells are liable to burst. In most scenarios associated with allegations of CW use, concentrations of free metabolites are likely to be higher in urine. Of much greater importance in blood are adducts with proteins, and these are described in Part B.

1.4 Analytical Methods

Most metabolites are small, relatively polar molecules that can be analyzed by gas chromatography/mass spectrometry (GC/MS), usually after derivatization, or underivatized by liquid chromatography/mass spectrometry (LC-MS). With most of the mass spectrometry (MS) instruments available, analysis is usually performed using the sensitive selected ion monitoring (SIM) mode. In most cases of allegations of use, analytes are unlikely to be present at concentrations that allow good quality full scan MS data to be obtained, although some modern ion trap and time of flight instruments may have sufficient sensitivity. In the prolonged later phase of excretion of urinary metabolites, single stage MS may not provide sufficiently low limits of detection, and gas chromatography (GC) or liquid chromatography (LC) combined with tandem MS (MS/MS) is usually required, using multiple reaction monitoring (MRM). MS/MS methods are preferable irrespective of the concentration of analyte as they generally provide a greater degree of specificity (unless full scan electron impact (EI) data can be obtained). Conversion to perfluorinated derivatives and analysis using negative ion chemical ionization/mass spectrometry (NICI/MS) has provided the most sensitive methods of analysis for polar metabolites.

Experience has shown that in allegations of CW use, particularly in remote conflicts, samples may be collected several days or even weeks after the event, for example, after casualties have been moved

to hospitals well away from the conflict. In these situations, covalent adducts with biological macromolecules, such as proteins and DNA, offer potentially longer-living biological indicators. These are discussed in Part B.

This paper will review the known metabolic pathways of CW agents, excretion profiles where these have been measured, and methods for the analysis of metabolites in urine or blood. Examples are provided of detection in cases of human exposure. The review focuses mainly on sulfur mustard and nerve agents that represent the greatest global CW threat, and for which most analytical methods have been developed.

2 SULFUR MUSTARD

Sulfur mustard remains one of the most important CW agents, despite the fact that it was first used more than 85 years ago. It was used to great effect in WW I, and stockpiled extensively but not used in WW II. More recently, it was used in the Iraq–Iran conflict, during which a number of biomedical samples were collected. Although not a lethal agent in most circumstances, it causes severe blistering by skin contact and its vapor causes lesions of the eyes and lungs. The physical properties of sulfur mustard are advantageous for a CW agent, and it is one of the easiest agents to produce. Its mechanism of action with regard to vesicancy is unknown but is assumed to result from its reactions with macromolecules.

2.1 Distribution and Metabolism

2.1.1 Distribution

The distribution and rate of elimination of sulfur mustard is partially dependent on the route of exposure and the dose received. Studies with radiolabeled (^{35}S) agent showed that typically 50–80 % of an absorbed dose is eliminated in urine, mostly within the first 3 days ^(2,3). Hambrook *et al.* ⁽²⁾ showed that >70 % was excreted in urine following intravenous (i.v.) or intraperitoneal (i.p.) injection in rats, somewhat less (50–70 %) following cutaneous exposure to vapor. The lower percentage following cutaneous exposure resulted from retention in the

skin. Urinary excretion of ^{35}S had a half-life of ~ 1.4 days. Much lower amounts of radioactivity were excreted in feces, typically 5–15 % of the dose, the higher amount after cutaneous application. It was also shown that ^{35}S persisted in blood for more than 6 weeks, associated mainly with the hemoglobin ⁽³⁾. Radioactivity was rapidly distributed throughout the tissues following i.v. administration in the rat ⁽⁴⁾.

2.1.2 Metabolism

Sulfur mustard possesses two electrophilic carbon atoms and its chemistry and metabolism are dominated by their reactions with nucleophiles. In most environments, nucleophilic reactions proceed by an internal $\text{S}_{\text{N}}1$ type mechanism, via the episulfonium ion shown in Figure 2. Formation of the episulfonium ion is rate-limiting and occurs rapidly in polar solvents ⁽⁵⁾. Sulfur mustard also possesses an electron-rich sulfur atom that reacts with electrophilic species such as oxidizing agents; it participates in the formation of the episulfonium ion. In a competitive environment, the episulfonium ion reacts preferentially with 'soft' nucleophiles, such as the thiol function of the cysteine residue in the tripeptide glutathione. Glutathione is relatively abundant in blood and is part of the body's defence mechanism against alkylating agents. Under physiological conditions, reaction with glutathione competes with the most abundant nucleophile water. The metabolism of sulfur mustard is therefore dominated by what are probably chemical (as opposed to enzyme mediated) reactions with glutathione and water, accompanied by metabolic oxidation of the sulfur atom to sulfoxide or sulfone. The various permutations of these reactions (on one or both carbon atoms), combined with divergent metabolic pathways for the reaction products with glutathione, lead to the formation of a large number of metabolites. Mustard also reacts with various sulfhydryl, hydroxyl, carboxyl, and amino groups on proteins and DNA. These are discussed in Part B.

Two conflicting metabolism studies were undertaken on sulfur mustard before the advent of modern mass spectrometry. In both studies, ~ 90 % of the dose administered to rats was excreted as urinary metabolites over 5 days, mostly within the first 48 h. Metabolites were tentatively identified by paper chromatography and inverse dilution assay with synthetic compounds. In the first study, in which ^{35}S -mustard was administered i.v. ⁽⁶⁾, the major metabolites were tentatively identified as the bis-*S*-glutathione conjugate, formed by the reaction of one molecule of sulfur mustard with two molecules of glutathione, plus thiodiglycol (TDG) from simple hydrolysis. The apparent lack of hepatic metabolism in this study was surprising. Some differences were observed in the metabolite profiles obtained from rats and terminal cancer patients. Using a much lower dose, a greater proportion of radioactivity was retained in cancer patients. In a second study using i.p. administration, hepatic metabolism was much more evident; the major metabolite being tentatively identified as the bis-*S*-(*N*-acetylcysteine) (or bis-mercapturic acid) conjugate of mustard sulfone, formed by the expected metabolism of the bis-*S*-glutathione conjugate ⁽⁷⁾. Also detected was a very polar metabolite (probably thiodiglycol sulfoxide (TDGO), see below), which was also formed after the administration of TDG.

Unequivocal identification of the bis-(*N*-acetylcysteine) conjugate (1) (Figure 3) was later made by Black *et al.* ⁽⁸⁾, also using i.p. administration in the rat. Repetitive LC was used to isolate metabolites and identification was by MS/MS, using desorption chemical ionization, and comparison with synthetic samples. Also identified at lower concentration, was the analogous conjugate of mustard sulfoxide, plus three mono-conjugates derived from the reaction of mustard with one molecule of glutathione. The metabolites identified from the glutathione pathways are shown in Figure 3. The most interesting metabolites identified were (2) and (3), derived from further metabolism of the bis-glutathione conjugate

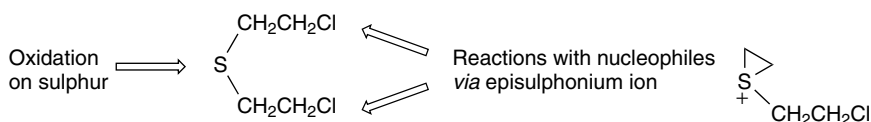


Figure 2. Chemical reactivity of sulfur mustard

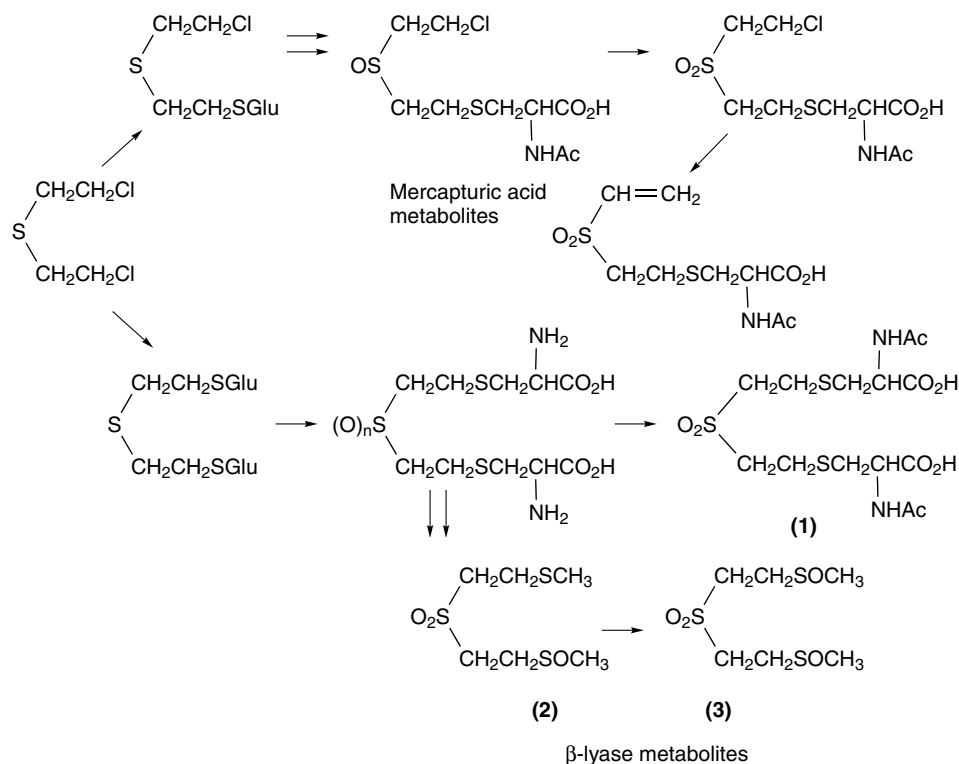


Figure 3. Metabolites of sulfur mustard identified by mass spectrometry, derived from an initial reaction with glutathione and metabolized by divergent mercapturic acid and β -lyase pathways ⁽⁸⁾

by the β -lyase pathway, which proceeds through cleavage of the S—C bond in the cysteine moiety followed by *S*-methylation ⁽⁹⁾. As with the earlier studies, a large number of metabolites were evident from LC radiochromatograms, and undoubtedly a number of additional metabolites could be identified using modern LC/MS techniques. In particular, no mono-*N*-acetylcysteine conjugates derived from hemi-mustard (i.e. containing a $\text{CH}_2\text{CH}_2\text{OH}$ moiety) were isolated but these are expected to be significant metabolites.

Metabolites derived from hydrolysis in the rat were TDG (4) and its sulfoxide TDGO (5), the former being a minor metabolite and the latter a major one. Also identified as a very minor metabolite, was the much less reactive mustard sulfoxide. These are shown in Figure 4.

Urinary excretion profiles were measured for TDG, TDGO, and β -lyase metabolites in the rat after percutaneous administration ⁽¹⁰⁾. Levels of TDG increased up to 10-fold by treatment of the urine

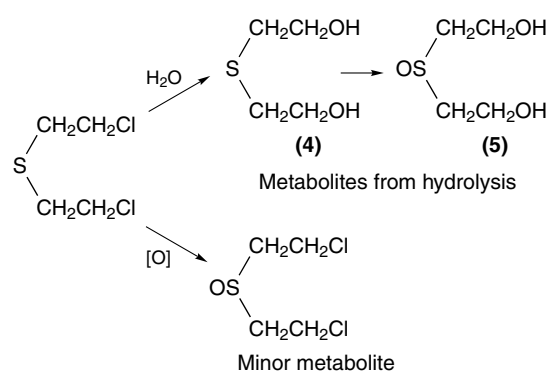


Figure 4. Metabolites of sulfur mustard identified by mass spectrometry, derived from hydrolysis and oxidation ⁽⁸⁾

with hydrochloric acid, indicating the presence of acid labile conjugates; TDGO levels rose by only a small amount ($\sim 30\%$) after acid treatment. Levels of TDGO were consistently several times higher than those of TDG. Free TDG, TDG liberated by

acid treatment, and TDGO excreted over 8 days accounted for <0.3 %, 1–1.5 %, and 3.4–4.3 % of the applied dose respectively. In a separate series of experiments, the hydrolysis products were detected as the single analyte TDG, and the β -lyase metabolites as a single reduced analyte (see below). Excretion of hydrolysis products over 8 days accounted for 3.7–13.6 % of the applied dose and β -lyase metabolites 2.5–5.3 % (there was considerable variation between animals). The excretion of β -lyase metabolites showed a much sharper decline than hydrolysis products (Figure 5), suggesting that the prolonged excretion of hydrolysis products results from TDG being slowly liberated from adducts with macromolecules (see Part B).

One aspect of the metabolism of sulfur mustard that appears to be unresolved is the relative importance of TDG and TDGO as excretion products in man. In metabolism studies in rats, TDGO was present in much higher concentrations than TDG. Furthermore, when ^{35}S $^{13}\text{C}_4$ -TDG was administered (i.p.) to rats, approximately 90 % of the

administered dose was excreted in the 0–24 h urine, mostly (≥ 90 %) as TDGO ⁽¹¹⁾. Thiodiglycol sulfone, *S*-(2-hydroxyethylthio)acetic acid, and *S*-(2-hydroxyethylsulfenyl)acetic acid were identified as minor metabolites resulting from oxidation on sulfur or carbon. Only 0.5–1 % of the TDG was excreted unchanged. Black *et al.* ⁽¹²⁾ found the sulfoxide to be the prevalent form in cases of human exposure, but Jakubowski *et al.* ⁽¹³⁾ detected quite high concentrations of TDG following an accidental laboratory exposure to sulfur mustard. As will be discussed below, TDG and TDGO are less than ideal as biological indicators of poisoning because true blanks in human urine cannot be obtained.

Two additional urinary excretion products have been reported for sulfur mustard, both presumably derived from an initial reaction with a macromolecule. N7-(2-Hydroxyethylthioethyl)guanine (**6**) was identified in the urine of guinea pigs treated with sulfur mustard (1 mg/kg, i.v.). This metabolite is derived from the breakdown of alkylated DNA (see Part B) ⁽¹⁴⁾. Excretion was maximal during the first 2–3 h (50 ng/ml), decreasing to 10 ng/ml in the interval 34–48 h. The imidazole derivative (**7**) was identified in plasma and urine following percutaneous administration of sulfur mustard in the pig ⁽¹⁵⁾. This metabolite was presumed to be derived from an initial reaction with a protein histidine residue.

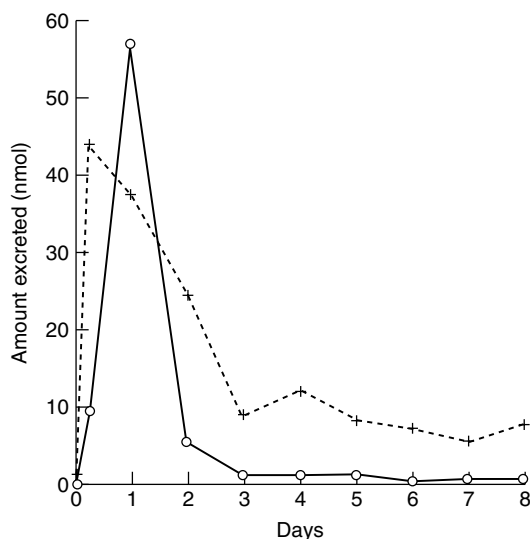
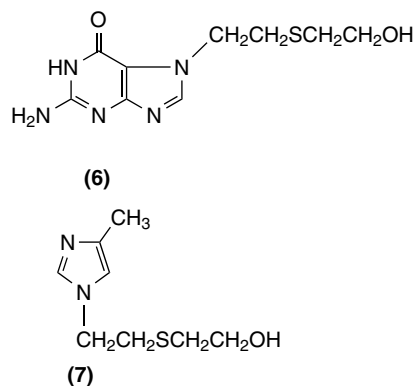


Figure 5. Urinary excretion profiles of metabolites derived from hydrolysis and the β -lyase pathway in the rat following cutaneous application of sulfur mustard (dose = $2\ \mu\text{mol}/\text{animal}$, data points mean of 4 animals; dotted line = hydrolysis products, solid line = β -lyase metabolites). (Reproduced from the Journal of Analytical Toxicology by permission of Preston Publications A Division of Preston Industries, Inc.)



2.1.3 Nonmetabolized Sulfur Mustard

Although an absorbed dose is expected to be fully metabolized, sulfur mustard is a highly lipophilic

molecule that will partition into fatty tissues. Surprisingly high concentrations of sulfur mustard were reported in various organs and tissues, particularly abdominal fat, removed *postmortem* from a lethal casualty of sulfur mustard poisoning ⁽¹⁶⁾. This subject had absorbed a large dose of agent. A biomedical sample that should not be overlooked is hair. A United Nations investigation (1986) reported the detection of unchanged sulfur mustard in hair from an alleged casualty of mustard poisoning ⁽¹⁷⁾. In the laboratory of one of the authors, some evidence was found to suggest that residues of sulfur mustard might become trapped within the hair matrix. A sample of human hair was exposed to sulfur mustard vapor and thoroughly extracted with solvent. It was then digested with the enzyme Pronase in the presence of dithiothreitol (which breaks disulfide linkages) in an effort to detect covalently bound residues. Surprisingly, mustard adducts with the dithiothreitol were observed.

2.2 Analytical Methods for Sulfur Mustard and its Metabolites

Analytical methods have been reported for unchanged agent and six of the urinary excretion products described above. These are: TDG, TDGO, the bis *N*-acetylcysteine conjugate (**1**), two β -lyase metabolites (**2**) and (**3**), and the guanine adduct (**6**). These methods have been applied to animal and/or human exposures to sulfur mustard.

2.2.1 Unmetabolized Agents

Trace analysis of sulfur mustard is relatively simple, although it is unlikely to be present in biomedical samples collected in most scenarios. The agent can be extracted from samples, for example, homogenized tissues ⁽¹⁶⁾ or blood ⁽¹⁸⁾, with organic solvents such as dichloromethane or ethyl acetate. Simple cleanup can be afforded using a solid-phase extraction (SPE) cartridge. Analysis can be performed by single stage GC/MS (with SIM) or by GC/MS/MS (with MRM), using a nonpolar capillary column.

2.2.2 Thiodiglycol

TDG, and particularly the more polar TDGO, are challenging metabolites to analyze in complex

aqueous matrices. TDG can be analyzed by GC/MS or LC/MS without derivatization, but these methods generally lack sensitivity. For limits of detection in the low parts per billion (ppb) range, thiodiglycol must be isolated from urine or blood in a nonaqueous solvent, derivatized, and analyzed by GC/MS or GC/MS/MS. Derivatives commonly used for environmental analysis are the trimethylsilyl (TMS) and *tert*-butyldimethylsilyl (TBDMS) ethers ⁽¹⁹⁾. These may be applicable to the analysis of biomedical samples collected soon after a relatively high exposure to sulfur mustard, but they are not well suited to analysis at low ppb levels. For biomedical sample analysis, pentafluorobenzoyl and heptafluorobutyryl esters have been used. Black and Read ^(20,21) analyzed TDG as its bis-pentafluorobenzoyl derivative using NICI/MS or NICI/MS/MS for detection. Urine, blood, or plasma, with ²H₄-TDG added as internal standard, was loaded onto a solid absorbent tube (which provides a large surface area for liquid-liquid extraction) and extracted with ethyl acetate. The extracts were cleaned up on Florisil (urine) or C18 (blood, plasma). After concentration, the dried residues were derivatized with pentafluorobenzoyl chloride (pyridine, ambient temperature, 5 min). The negative ion chemical ionization (NICI) mass spectrum of TDG-bis-pentafluorobenzoate shows only one significant ion, the molecular ion at *m/z* 510. With the ion current concentrated in this single high mass ion (plus isotope peaks), the method is very sensitive; detection limits were ~1 ng/ml. A disadvantage is that it is limited for confirmatory analysis, and a second analysis on a different GC column would be recommended in cases of allegations of CW use (and detection of a second biological indicator). A small increase in selectivity can be achieved using GC/MS/MS ⁽²¹⁾. Collision induced dissociation (CID) of *m/z* 510 gave only nonspecific product ions [C₆F₅CO₂]⁻, *m/z* 211, and [C₆F₅]⁻, *m/z* 167, derived from the pentafluorobenzoyl moiety. However, significantly cleaner chromatograms were obtained by monitoring the parent ion of *m/z* 167, compared to single stage MS using SIM. For standards, 0.2 pg injected could easily be detected, indicating a theoretical limit of detection (LOD) of ~0.1 ng/ml, making it the most sensitive method to have been reported for TDG. The true detection limit in urine could not be determined because of very low

background levels (usually <1 ng/ml) of TDG. A second disadvantage of the method is that pentafluorobenzoyl chloride does not give a clean reaction product, and the procedure is only suitable for use with NCI/MS. A recent modification, for analyzing TDG released by hydrolysis from macromolecules, includes a partial cleanup on C18 after derivatization ⁽²²⁾.

Jakubowski *et al.* ⁽²³⁾ analyzed TDG in urine as its bis-heptafluorobutyl (HFB) derivative. $^2\text{H}_8$ -TDG was added as internal standard, and the urine incubated for 1 h with glucuronidase with sulfatase activity to hydrolyze any conjugates present. The urine was then adjusted to pH 3–4, concentrated to dryness, reconstituted in ethyl acetate, and derivatized with heptafluorobutyric anhydride (60°C , 1 h). The HFB derivative produced analytically useful fragment ions at m/z 300 and 301 (70 and 50 % relative abundance), resulting from the loss of the $\text{C}_3\text{F}_7\text{CO}_2$ moiety. Using SIM, TDG could be detected in urine down to concentrations of 1 ng/ml. The advantage of this method is that it is usable on basic benchtop GC/MS systems. A disadvantage is the need to concentrate urine to dryness, which is time consuming, and a potential source of error through evaporative losses and residual traces of water interfering with the derivatization. The HFB derivative also shows promise under positive chemical ionization (CI) conditions ⁽²⁴⁾.

Wils *et al.* ^(25,26) previously reported an entirely different approach to TDG analysis. TDG in urine was converted back to sulfur mustard by treatment with concentrated HCl. The sample treatment is less straightforward than the methods described above, but analysis as sulfur mustard is facile. Urine, plus $^2\text{H}_8$ -TDG as internal standard, was cleaned up by elution through two C18 cartridges. Concentrated HCl was added and the sample stirred and heated at 120°C . Nitrogen was blown over the solution and sulfur mustard isolated from the headspace by adsorption onto Tenax-TA. The method was used to detect TDG in urine from casualties of CW attacks (see below). A disadvantage of this method is that it may convert metabolites other than TDG to sulfur mustard. This is supported by the detection of relatively high levels of analytes in urine from control subjects. Vycudilik ⁽²⁷⁾ used a similar procedure, but recovered the mustard by steam distillation and extraction.

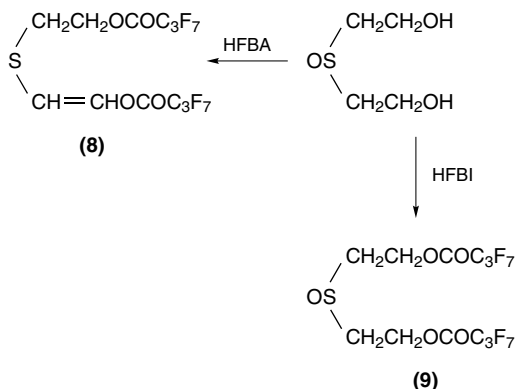
2.2.3 Thiodiglycol Sulfoxide

Trace analysis of TDGO is more difficult than TDG due to the high polarity of the sulfoxide moiety. TDGO is considerably more polar than TDG and the corresponding sulfone, making selective isolation from aqueous matrices difficult. A cleanup is problematic because it is highly retained on normal phase silica, requiring very polar solvents for elution, and is not retained under reversed phase conditions. TDGO can be analyzed by LC/MS but, as with TDG, detection limits are modest. Derivatization of TDGO for GC/MS analysis can be problematic because the sulfoxide oxygen is an additional nucleophilic site for reaction.

Black and Read ⁽²⁸⁾ developed a procedure that extracted TDGO with ethyl acetate-methanol (100:7) after absorption of urine, with $^2\text{H}_4$ -TDGO added as an internal standard, onto a solid absorbent. The extract was eluted through a Florisil cartridge, concentrated to dryness, and derivatized with pentafluorobenzoyl chloride as for TDG. This procedure is not ideal because the high polarity of the solvent required for extraction produces an extract containing high levels of extraneous materials. A second disadvantage is that the derivative formed is the same as that produced from TDG, that is, the sulfoxide function is reduced. This makes it difficult to distinguish the sulfoxide from TDG at trace levels other than by selective extraction. The detection limit is similar to that for TDG, but more extraneous peaks are observed in the chromatogram because of the solvent required for extraction.

Derivatization of TDGO is complicated because three major types of derivatives are formed, depending on the reagent and conditions ⁽²⁴⁾. These result from selective derivatization of the hydroxyl groups with preservation of the sulfoxide function, reduction to the corresponding thiodiglycol derivative, and Pummerer-type rearrangement to derivatives or elimination products of 1-hydroxythiodiglycol. For example, the reaction with heptafluorobutyric anhydride gave a derivative tentatively identified as (8), derived from a Pummerer-type rearrangement and elimination (it is not yet clear whether the elimination occurs during the derivatization or in the hot injector). A similar product was obtained with trifluoroacetic anhydride. Heptafluorobutylimidazole (HFBFI) gave the sulfoxide derivative (9) as the major product. Reduction to the TDG derivative

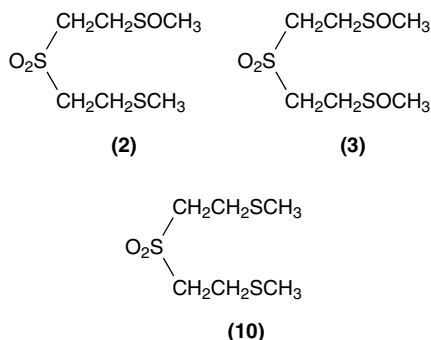
was observed with heptafluorobutyl chloride and pentafluorophenyldimethylsilyl chloride. These derivatization reactions are currently being evaluated for biomedical sample analysis.



An alternative approach to TDGO analysis is to reduce it to TDG by treating urine with titanium trichloride⁽²⁸⁾. This provides a convenient procedure for the combined analysis of hydrolysis products and β -lyase metabolites (see below).

2.2.4 β -Lyase Metabolites

The two metabolites identified in the rat, derived from the β -lyase pathway, (2) and (3), have one and two sulfoxide groups respectively. These promote elimination reactions on hot surfaces in the GC/MS, as well as imparting higher polarity to the molecules. Reduction of the sulfoxide groups to sulfide with titanium trichloride⁽²⁹⁾ produces a single analyte (10), which is efficiently extracted from urine on a C8 SPE cartridge, and gives a sharp GC peak. The hexadeuterated bis-SOC[²H]₃ analogue of (3) was added to urine as an internal standard.



Single stage GC/MS analysis, using positive ammonia CI and SIM of $[M + NH_4]^+$, m/z 232, gave a detection limit of 2 ng/ml⁽²⁹⁾. GC/MS/MS provided a much lower detection limit (0.1 ng/ml), monitoring the fragmentation m/z 232 \rightarrow $[MeSCH_2CH_2]^+$, m/z 75, resulting from a substantial reduction in the chemical background⁽²¹⁾. The chromatograms obtained from single-stage and tandem MS are compared in Figure 6. This improved LOD allowed the detection of β -lyase metabolites in the urine of seven CW casualties (see Section 2.3.3). The method was originally developed for use on a triple sector quadrupole mass spectrometer, and a detection limit of 0.1 ng/ml was easily achievable. However, transfer of the method to a benchtop-GC-ion trap MS system has resulted in higher and rather inconsistent limits of detection (0.5–1 ng/ml). A possible reason is that the analyte, which contains three sulfur atoms, is sensitive to hot surfaces.

The use of titanium trichloride for the reduction of sulfoxide groups allows a combined approach to the analysis of urine for metabolites derived from hydrolysis and the β -lyase pathway, as shown in Figure 7^(10,21). Urine is treated with titanium trichloride, divided into two aliquots, and analyzed for TDG and the reduced β -lyase product (10) as described above.

An alternative to GC/MS/MS of the reduced product (10) is direct LC/ESI/MS/MS of (2) and (3). The analytes can be isolated from urine by SPE on a hydroxylated polystyrene-divinylbenzene (PS-DVB) polymeric cartridge. Using a sensitive triple sector quadrupole LC/MS/MS system, detection limits of 0.1 ng/ml have been achieved using positive ESI and MRM⁽³⁰⁾.

2.2.5 Bis-(N-Acetylcysteine) Conjugate of Mustard Sulfone

Attempts to develop a GC/MS method for this metabolite were unsuccessful, no doubt because of thermal instability. An LC/MS/MS method using electrospray ionization, after derivatization to the dimethyl ester, gave a modest detection limit of 25 ng/ml, again probably due to poor thermal stability, in this case, in the electrospray ion source⁽¹²⁾. A substantial improvement has recently been achieved (detection limit 1 ng/ml) using LC/ESI/MS/MS without derivatization⁽³¹⁾. Concentration from acidified urine was achieved on

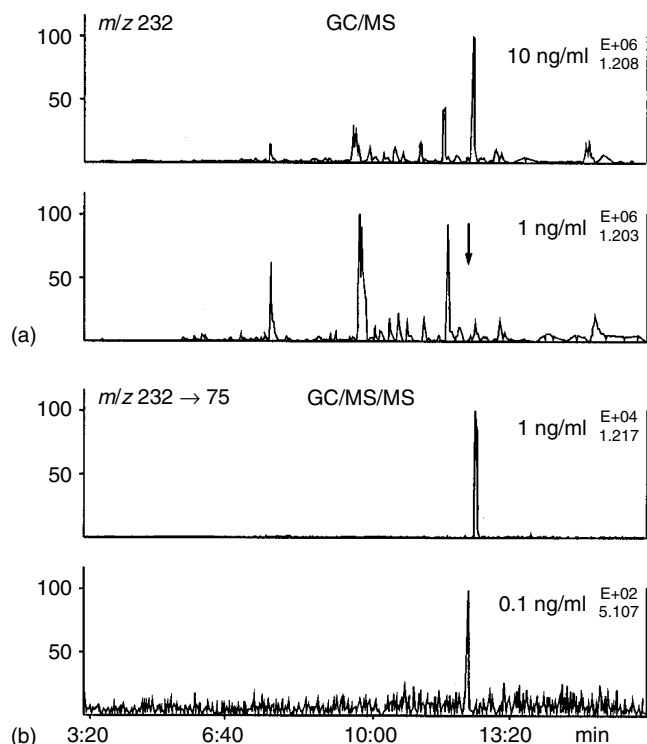
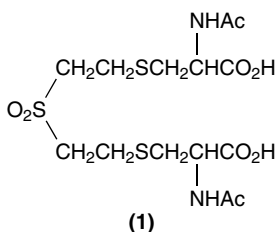


Figure 6. GC/MS/SIM (upper) and GC/MS/MS/MRM (lower) chromatograms for reduced β -lyase metabolites, showing a >50 -fold improvement in signal-to-noise ratio using GC/MS/MS. (Reprinted from Journal of Chromatography B, **665**, R.M. Black and R.W. Read, Improved methodology for the detection and quantitation of urinary metabolites of sulphur mustard using gas chromatography-tandem mass spectrometry, pp. 97–105 (1995), with permission from Elsevier)

a polymeric SPE cartridge and detection was by negative ion ESI using MRM.



2.2.6 N7-(2-Hydroxyethylthioethyl)guanine

The guanine adduct (**6**) can be isolated from urine by SPE on C18. GC/MS analysis of the adduct was problematic. Derivatization with heptafluorobutyric anhydride and pentafluorobenzoyl bromide were unsuccessful and the TBDMS derivative

had poor GC properties. A sensitive method was developed for the underivatized compound using LC/ESI/MS/MS, monitoring the fragmentation MH^+ , m/z 256 \rightarrow $[\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{OH}]^+$, m/z 105. The detection limit was 8 pg injected (S/N 5:1), 0.2 ng/ml in urine ⁽¹⁴⁾.

2.3 Application to Human Casualties

Most of the analytical methods reported above have been successfully employed in cases of human poisoning, either in casualties of the Iraq–Iran and Iraq–Kurd conflicts, or in cases of accidental poisoning.

2.3.1 Unmetabolized Agent

As part of a UN investigation into the use of chemical weapons in the Iraq–Iran war ⁽¹⁷⁾, sulfur

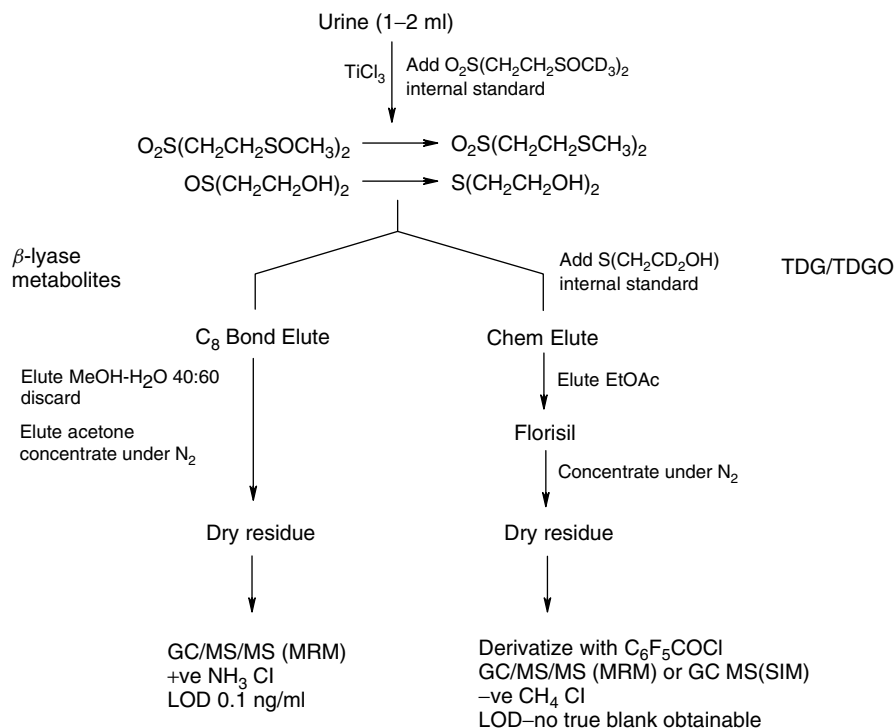


Figure 7. Scheme for the combined analysis of urine for sulfur mustard metabolites derived from hydrolysis and the β -lyase pathway

mustard was detected in a dichloromethane extract of the hair of one casualty, but not in another. The sample was believed to have been collected soon after the exposure. Identification was by full scan GC/MS, the concentration estimated as 0.5–1 $\mu\text{g/g}$ (ppm). Drasch⁽¹⁶⁾ reported surprisingly high concentrations of sulfur mustard in tissues removed postmortem from a CW casualty who died seven days after exposure. Qualitative analysis was performed using full scan GC/MS and quantitative analysis by electrothermal atomic absorption spectrometry of a mustard-gold complex. Concentrations of mustard reported in various organs (lung, kidney, brain etc.) ranged from 0.8–10.7 $\mu\text{g/g}$, and in fatty tissues including skin up to 15.1 $\mu\text{g/g}$. Concentrations of 1.1 $\mu\text{g/ml}$ were reported in blood but none was detected in urine or blister fluid. These results suggest that mustard sequestered in fatty tissues may remain in the body longer than previously suspected from animal studies, though the concentrations in blood are surprising.

2.3.2 TDG and TDGO

Wils *et al.*^(25,26) reported the detection of TDG in urine from Iranian casualties, by the conversion of TDG back to sulfur mustard with HCl. The alleged attacks occurred in 1984 and 1986, and samples were collected after casualties had been removed to hospitals in Utrecht and Ghent. Urine was immediately frozen on collection for transport to the analytical laboratory and then stored at -23°C . Concentrations of TDG detected in urine collected 5–10 days after the alleged attack were mostly in the range 5–100 ng/ml , but in one casualty 330 ng/ml (this patient died one day after admission). A second set of samples collected from one of the groups 18 days after the reported exposure showed very low levels of the analyte (in the range 3–8 ng/ml). A complicating factor was the presence of low levels of the analyte in control urine samples. In most cases, these were in the range 1–12 ng/ml . However, a concentration of 55 ng/ml was detected

in one sample from a control patient in the first investigation, and 21 ng/ml was detected in a control sample in the second. These values were closer to those detected for TDGO in normal urine ⁽²⁸⁾, and suggest that the method also converts other urinary excretion products to sulfur mustard. When TDG was analyzed directly as its bis-pentafluorobenzoyl or bis-heptafluorobutyryl derivative, concentrations found in control samples were generally <1 ng/ml, although no true blank could be obtained ^(12,20,23).

TDG and TDGO (after reduction to TDG) were detected retrospectively in urine samples taken from three of the same casualties, collected 8 days after the exposure and after storage at -20 °C for 5 years. Concentrations were in the range 27–72 ng/ml, compared to a concentration of 11 ng/ml in a control sample. In two casualties accidentally exposed to sulfur mustard from a WW I munition, concentrations in urine collected 2–3 days after the exposure were TDG 2 ng/ml (in both), TDGO 69 ng/ml and 54 ng/ml (mean of 3 determinations) ⁽¹²⁾. These are shown in Table 1.

Jakubowski *et al.* ⁽¹³⁾ were able to monitor the excretion of TDG in a subject accidentally exposed to sulfur mustard in a laboratory. The casualty developed blisters on hands and arms (< 1 % of body area) and erythema on his face and neck (< 5 % of body area). Urine was collected over a 10-day period. A maximum excretion rate of TDG of 20 ug per day was observed between days 3 and 4, the highest concentration being ~65 ng/ml. It was noted that the total amount of urine produced for analysis during the first three days was low. Concentrations > 10 ng/ml were detected in urine for 7 days after the exposure. The half-life of excretion was estimated as 1.18 days. The total amount of TDG excreted over the 10-day period was 243 ug. There was mass spectrometric evidence of oligomers of TDG (e.g.

(HOCH₂CH₂SCH₂CH₂)₂O) in a sample of blister fluid. A crude estimate of the cutaneous exposure was ≥49 mg.

Neither TDG or TDGO are ideal biological markers of exposure because of the very low concentrations present in the urine and blood of normal subjects. TDG appears to be present in urine usually at levels <1 ng/ml, but higher concentrations (up to 16 ng/ml) have been detected in blood ⁽²⁰⁾. TDGO was detected at concentrations mainly in the range 1–10 ng/ml in normal human urine, but in one subject it was as high as 36 ng/ml ⁽²⁸⁾. The source of these background levels is unknown but may be dietary.

2.3.3 *β*-Lyase Metabolites

β-Lyase metabolites have been detected in urine samples from nine subjects. Five were from Iranian casualties (three of which were also analyzed for TDG and TDGO, see above), two from Kurdish casualties, and two from the subjects accidentally exposed from a WW I munition. The urine from one of the Iranian casualties contained exceptionally high levels of *β*-lyase metabolites (~220 ng/ml). This subject died one day after admission to hospital, and is believed to be the casualty reported by Wils *et al.* ⁽²⁶⁾ to be excreting very high levels of TDG. Much lower levels (0.5–5 ng/ml) were found in urine from the other four Iranian casualties. Samples from two Kurdish casualties of mustard poisoning, collected 13 days after the reported attack, contained concentrations of *β*-lyase metabolites close to the LOD (0.1–0.3 ng/ml). GC/MS/MS MRM chromatograms are shown in Figure 8. These analyses clearly demonstrated the advantages of sub-ng/ml detection limits, as these low concentrations would not have been detected using single stage GC/MS. The

Table 1. Concentrations of metabolites found in the urine of two subjects accidentally exposed to sulfur mustard from a WW I munition

Metabolite	Amount detected (ng/ml)		
	Subject 1	Subject 2	Control
TDG (free and conjugated)	2,2	2,2	<1
TDGO	70, 66, 70	42, 42, 50	5, 5
<i>β</i> -Lyase metabolites	43, 42, 42	56, 57, 55	<0.1
Mustard sulfone bis(<i>N</i> -acetylcysteine) conjugate ^a	~1	~1	<1

^aAnalyses performed after a further 10 years storage at ≤ -15 °C

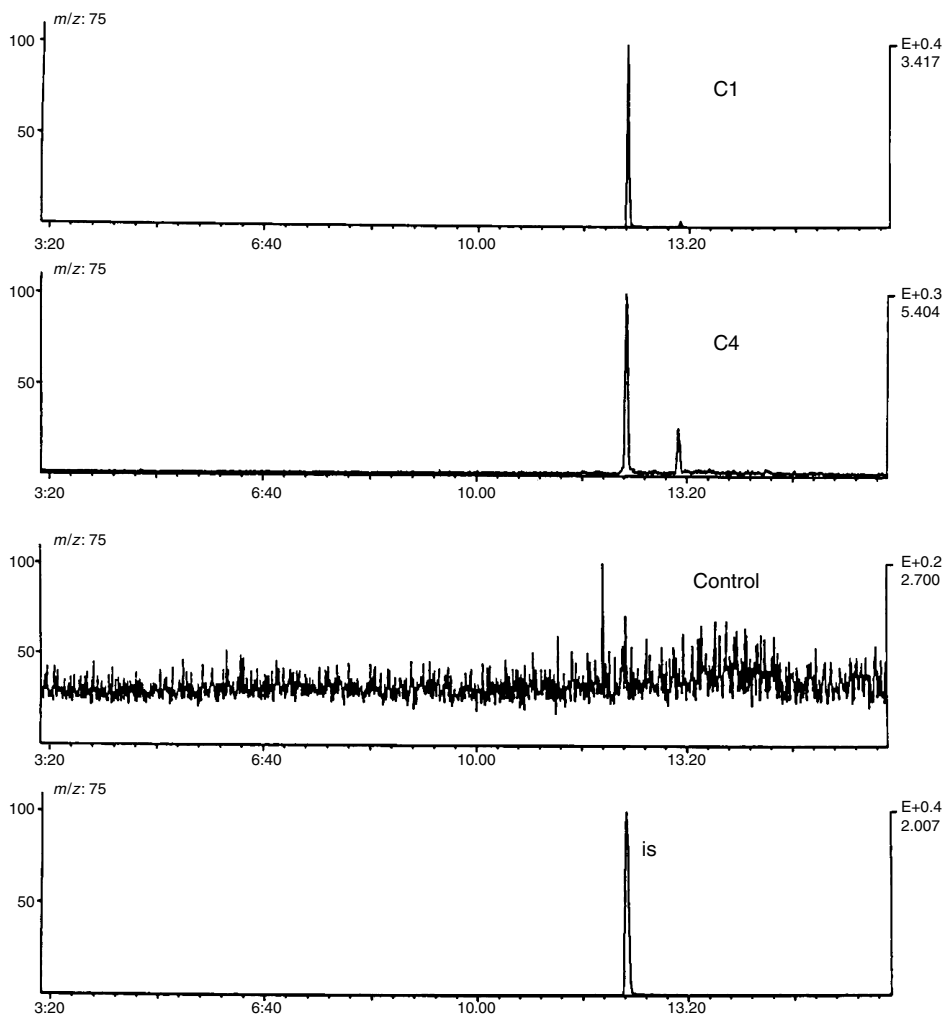


Figure 8. GC/MS/MS/MRM chromatograms showing the detection of β -lyase metabolites in urine from two casualties of sulfur mustard poisoning (C1, ~ 220 ng/ml and C4, ~ 5 ng/ml), their absence in urine from an unexposed subject, and the internal standard (is). Samples C1 and C4 were from two Iranian subjects undergoing treatment in Ghent in 1984, collected 10 days after the exposure

analyses also demonstrated the advantages of β -lyase metabolites over hydrolysis products as unequivocal indicators of mustard poisoning. True blanks have always been obtained for β -lyase metabolites in control urine samples. The larger quantity of urine obtained from the two subjects accidentally exposed to sulfur mustard from a WW1 munition allowed a comparison of concentrations of β -lyase metabolites and hydrolysis products. The results are shown in Table 1. In both subjects substantial amounts of both types of metabolite were

excreted but true control blanks were only obtained with β -lyase metabolites. Very recently, the β -lyase metabolites in these samples were determined by LC/ESI/MS/MS; in both cases the disulfoxide (**3**) was present in greater concentration than the monosulfoxide (**2**)⁽³⁰⁾.

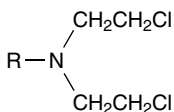
2.3.4 Bis-(*N*-Acetylcysteine) Conjugate of Mustard Sulfone

An initial analysis of urine from the two accidental casualties reported above, using LC-thermospray/

MS/MS, was negative for the bis-(*N*-acetylcysteine) conjugate (**1**). Very recently, these samples have been reanalyzed using the LC/ESI/MS/MS method reported in Section 2.2.5. Low concentrations (~ 1 ng/ml) were detected in both samples⁽³¹⁾. These are lower than would have been expected from animal studies, but the samples had been stored frozen for 12 years and thawed and refrozen on several occasions.

3 NITROGEN MUSTARDS

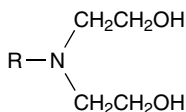
Three nitrogen mustards (**11a–c**) are included in Schedule 1 of the CWC. Although they are potent vesicants and simple to produce, nitrogen mustards are generally regarded as less effective than sulfur mustard as CW agents. They have accordingly attracted much less attention and few analytical methods have been reported for their analysis. HN-2 has been used as an anticancer agent and some metabolism studies have been reported in this context.



(**11a**) HN-1, R = Et

(**11b**) HN-2, R = Me

(**11c**) HN-3, R = CH₂CH₂Cl



(**12a**) R = Et

(**12b**) R = Me

(**12c**) R = CH₂CH₂OH

3.1 Metabolism

Nitrogen mustards are hydrolyzed to the corresponding ethanolamines (**12a–c**) in aqueous media and, like TDG, these are expected to be excretion products following exposure. No detailed mass spectrometric studies of the *in vivo* metabolism of nitrogen mustards have been reported. Lemire *et al.*⁽³²⁾ referred to unpublished studies that showed substantial amounts of free (unconjugated) *N*-ethyl and *N*-methyldiethanolamine were present in 24 h and 48 h urine from rats exposed to HN-1 and HN-2 respectively. *In vitro* studies have indicated that *N*-demethylation may be an important metabolic pathway for HN-2⁽³³⁾.

3.2 Analytical Methods

Lemire *et al.*⁽³²⁾ reported a quantitative method for the determination of *N*-ethyl- and *N*-methyldiethanolamine, the hydrolysis products of HN-1 and HN-2, in urine using LC/ESI/MS/MS on a triple sector quadrupole instrument. The analytes were concentrated from urine by SPE on a strong cation exchanger. In order to obtain good peak shapes, 73 % 3 mM ammonium hydroxide (pH 10.5) – 27 % methanol was used as the mobile phase for LC. Isotope dilution ([¹³C]₄-*N*-Me and *N*-Et diethanolamines) was used to compensate for inherent variabilities. Detection was by MRM, monitoring the transition MH⁺ → [MH-H₂O]⁺ for each analyte. The limits of detection were 0.4 ng/ml for *N*-ethyldiethanolamine and 1 ng/ml for *N*-methyldiethanolamine. An interferent was present in urine with similar retention time and nominal mass characteristics as *N*-methyldiethanolamine.

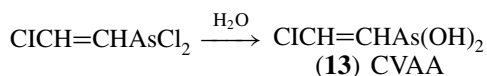
4 LEWISITE

Like sulfur mustard, lewisite is a potent vesicant but with greater volatility and a more rapid onset of action. Lewisite was first manufactured towards the end of the 1914–1918 war, but not in time to be used in that conflict. Unlike sulfur mustard and nerve agents, no human exposures to lewisite have been reported in which biomedical samples have been collected. Weapon grade lewisite is composed of ~ 90 % lewisite 1, ClCH=CHAsCl₂, up to ~ 10 % lewisite 2, (ClCH=CH)₂AsCl, and < 1 % of lewisite 3, (ClCH=CH)₃As, all predominantly as the trans isomers. In addition to being weaponized neat, lewisite was mixed with sulfur mustard to depress the freezing point of the latter and to impart a faster onset of effects. There remains a legacy of old and abandoned munitions, and former production sites.

4.1 Metabolism

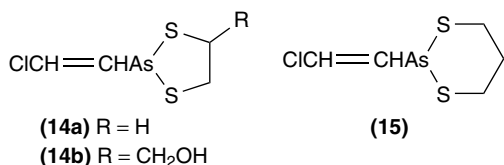
No detailed metabolism studies have been reported for lewisite. It is rapidly hydrolyzed to 2-chlorovinylarsonous acid (CVAA) (**13**) in the presence of moisture, and Waters and Williams reported that

CVAA is excreted in the urine of experimental animals⁽³⁴⁾. This has since been confirmed by modern analytical methods.



4.2 Analytical Methods

Trivalent arsenic forms strong bonds with sulfur, and thiols are therefore used for derivatizing both lewisite and CVAA, forming the same derivative⁽¹⁹⁾. Lewisite reacts with mono and dithiols; the reaction with dithiols occurring rapidly at ambient temperature. In a competitive environment, lewisite reacts almost exclusively with dithiols rather than monothiol⁽³⁵⁾. Three dithiols, 1,2-ethanedithiol, 1,3-propanedithiol and 2,3-dimercaptopropan-1-ol (British Anti-Lewisite, BAL) have been used for biomedical sample analysis of CVAA to form cyclic derivatives (14a, b) and (15). Unlike derivatization of TDG, CVAA can be derivatized directly in an aqueous solution. 2,3-Dimercaptotoluene, which has been used extensively for environmental analysis⁽¹⁹⁾, does not appear to have been used.



Jakubowski *et al.*⁽³⁶⁾ developed a GC/MS method for CVAA spiked into guinea pig urine using 1,2-ethanedithiol for derivatization, with phenyl arsine oxide as the internal standard. The same group later expanded the method to include atomic emission detection (AED)⁽³⁷⁾. CVAA was concentrated from urine (adjusted to pH 6 with 1 M HCl) by SPE on C18. After elution with methanol and concentration to dryness, the residue was reconstituted and derivatized with ethanolic 1,2-ethanedithiol. Detection was by GC combined with arsenic selective AED and by electron impact/mass spectrometry (EI/MS) using SIM. Ions monitored were the moderately intense M⁺ ion at *m/z* 228, an intense ion [M – C₂H₄]⁺, *m/z* 200, and a base

peak [AsS₂C₂H₄]⁺, *m/z* 167. The LOD was rather high at ~100 ng/ml. CVAA was detected in the urine of guinea pigs up to 24 h after exposure to lewisite (0.5 mg/kg s.c.). Excretion was rapid; a mean concentration of 3.5 ug/ml was detected in the 0–8 h urine, which had decreased to ≤100 ng/ml after 16–24 h.

Fidder *et al.*⁽³⁸⁾ used BAL to derivatize free CVAA in blood and urine, and to displace lewisite residues bound to a cysteine residue in hemoglobin (see Part B). Blood or urine was incubated with BAL (ambient temperature, overnight), with phenylarsine oxide-BAL derivative added as internal standard, and the dithiarsenoline derivative extracted using C18 SPE. The free hydroxyl on the resulting cyclic derivative (14b) was converted to its heptafluorobutyl derivative with heptafluorobutylimidazole (50 °C, 1 h, toluene); this was observable on the GC/MS chromatogram as a pair of diastereoisomers. This method was sensitive using GC/EI/MS SIM monitoring the molecular ion *m/z* 454 (20 pg injected); NICI was less suitable because of the absence of a pseudomolecular ion and the dominance of the nonspecific [C₃F₇CO₂][–] ion. The LOD was 10 nM (~2 ng/ml). CVAA could be detected in the urine of guinea pigs injected with 0.25 mg/kg lewisite only up to 12 h post exposure.

The most sensitive method for CVAA has recently been reported by Wooten *et al.*⁽³⁹⁾ using solid-phase microextraction to concentrate the derivatized analyte. Urine, with added ammonium acetate buffer and PhAsO as an internal standard, was derivatized directly with 1,3-propanedithiol and the derivative concentrated on a poly(dimethylsiloxane) (PDMS) solid-phase microextraction (SPME) fiber. Analysis was by automated GC/MS using SIM of the isotopic MH⁺ ions. An impressive detection limit of 7.4 pg/ml was reported, using a benchtop GC/MS system. The method was validated using spiked human urine.

5 ORGANOPHOSPHORUS NERVE AGENTS

Organophosphorus nerve agents are the most potent of the lethal CW agents declared to the OPCW. They were developed just before, during, and shortly after WW II, but were not used in that conflict.

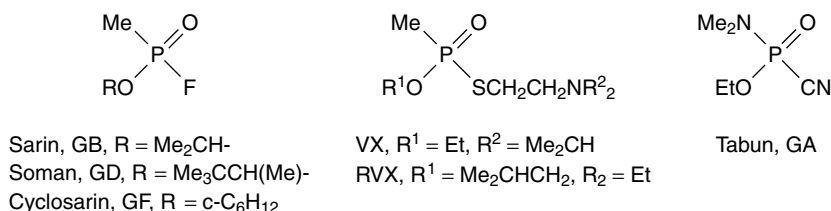


Figure 9. Chemical structures of nerve agents

Their use has since been confirmed in the Iraq–Iran conflict, against the Kurdish population in Iraq, and by terrorists in Japan. They can be divided into three structural classes (Figure 9): the alkyl methylphosphonofluoridates, usually referred to as G agents, for example, sarin (GB), soman (GD), cyclohexylsarin (GF); the V agents, VX (U.S.) and R-33 (Russia); and tabun (GA) ⁽⁴⁰⁾.

5.1 Distribution and Metabolism

The metabolism of nerve agents is much simpler than that of sulfur mustard. The major pathway for elimination is *via* enzyme-mediated hydrolysis by esterases, plus some chemical hydrolysis, as shown in Figure 10. In the case of the methylphosphonofluoridates and V agents, the major product is an alkyl methylphosphonic acid (alkyl MPA) (16). A small fraction of the nerve agent binds

covalently to cholinesterases, the biochemical target (see Part B). Detailed elimination studies have been reported only for the alkyl methylphosphonofluoridates. Shih *et al.* ⁽⁴¹⁾ determined the elimination kinetics of the main metabolites of sarin, soman, and cyclohexylsarin in the rat (0.075 mg/kg, s.c.). The agents were initially distributed rapidly throughout the tissues. Urinary excretion, as the alkyl MPAs, was the major route of elimination for all three compounds. Only traces of methylphosphonic acid (MPA), from further hydrolysis of the acids, were observed, although significant amounts were reported in the urine/plasma of casualties of Japanese terrorist attacks with sarin ^(42,43). Approximately 90 % of the applied dose of sarin and cyclohexylsarin was excreted in the urine within 24 h, with terminal elimination half-lives of 3.7 h and 9.9 h respectively. Soman was excreted at a slower rate, approximately 50 % within the first 24 h, rising to 62 % after 7 days. It showed a biphasic

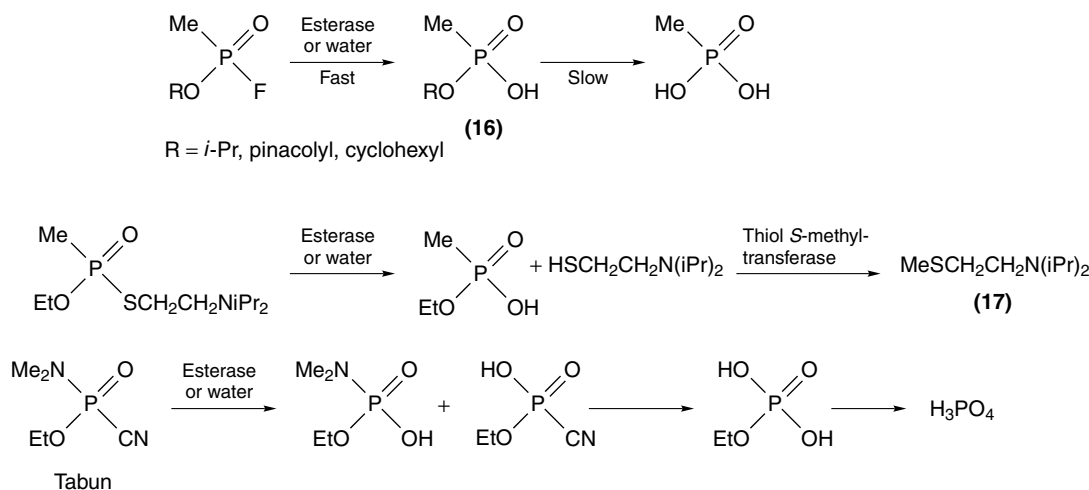


Figure 10. Metabolic and/or hydrolysis pathways for nerve agents

elimination with elimination half-lives of 3.6 h and 18.5 h. Benschop *et al.* ⁽⁴⁴⁾ have shown that the first phase of elimination of soman is due to enzymatic hydrolysis of the inactive P(+) isomer, whereas the slower phase is from the active P(−) isomer. The alkyl MPAs were also detected in blood, isopropyl (*i*-PrMPA) up to 14 h post exposure, cyclohexyl (cHexMPA) up to 2 days, and pinacolyl (PinMPA) up to at least 3 days ⁽⁴¹⁾.

VX appears to follow a similar pathway, the major metabolite being ethyl MPA (EMPA). An additional metabolite, derived from the diisopropylaminoethyl substituent, was identified in human plasma following an assassination with VX ⁽⁴⁵⁾. The sulfide (**17**), derived from enzymatic *S*-methylation of the hydrolysis product $\text{HSCH}_2\text{CH}_2\text{N}(i\text{-Pr})_2$, was identified in human serum by GC/MS after simple extraction. Experiments in rats confirmed the rapid metabolic formation of (**17**) from $\text{HSCH}_2\text{CH}_2\text{N}(i\text{-Pr})_2$ ⁽⁴⁶⁾. Identification of this metabolite distinguishes VX from the *O*-ethyl analogue of sarin.

5.2 Analytical Methods for Nerve Agents and Their Metabolites

5.2.1 Unmetabolized Agent

It is unlikely that the unchanged nerve agent would be detected in the blood or tissues of a casualty unless samples were collected very soon after the exposure. A number of methods have been reported for the analysis of nerve agents in blood, for application to animal studies. These involve simple liquid or SPE extraction, for example, using chloroform (sarin, soman) ⁽⁴⁷⁾, C18 SPE (sarin, soman) ^(48,49), ethyl acetate (VX) ⁽⁵⁰⁾, usually after precipitation of proteins, and analysis by GC/MS or gas chromatography/nitrogen–phosphorus detection (GC/NPD). Sarin bound to cholinesterase and displaced with fluoride ion was extracted by C18 SPE (see Part B) ⁽⁵¹⁾.

5.2.2 Alkyl Methylphosphonic Acids

(a) Overview

More than a dozen mass spectrometric methods have been reported for the trace analysis of alkyl MPAs in environmental and biomedical samples.

Methods based on derivatization and GC/MS/MS (or high resolution GC/MS) have provided the lowest limits of detection, although the latest generation of very sensitive LC/MS instruments may achieve comparable limits of detection (see an example below). TMS, TBDMS, and methyl esters are commonly used for GC/MS analysis of alkyl MPAs in environmental samples ⁽¹⁹⁾. Both types of silyl derivative were successfully used in the analysis of blood and urine samples from Japanese terrorist incidents, but many of the samples were collected within a few hours of the exposure. In cases of allegations of CW use, particularly in remote conflicts, samples are more likely to be collected many days after the alleged event, and much lower detection limits may be required. Another factor to consider is that casualties surviving an exposure to a nerve agent will probably have absorbed a much lower dose than the casualties who survived mustard poisoning, described in Section 2.3. As is the case with TDG, conversion of alkyl MPAs to perfluorinated derivatives (pentafluorobenzyl esters) and analysis by NICI GC/MS/MS has provided the lowest limits of detection ⁽¹⁹⁾.

Isolation from the matrix is a key stage in the analysis of alkyl MPAs and, in combination with subsequent derivatization, is a potential source of error. A number of procedures that isolate the acids using SPE have been reported for blood and urine. These retain the ionized acid by anion exchange or the unionized acid by hydrophobic interactions on C₂, C₈, C₁₈, or polymeric cartridges. Alternatively, liquid–liquid extraction can be used. Ideally, a single procedure is desirable that efficiently recovers the range of alkyl MPAs of interest, but the widely differing hydrophobic properties, for example, of EMPA and PinMPA, make this difficult. MPA is a particularly difficult acid to analyze at trace levels. An alternative to extracting the acids from the biological matrix is to remove as many extraneous materials as possible and then concentrate to dryness. Hydrophobic materials can be removed from urine at around neutral pH by elution through a C18 or polymeric cartridge, although losses of the more hydrophobic acids, PinMPA and cHexMPA, may occur. Inorganic cations or anions can be removed by selective ion exchange.

In our experience, GC/MS procedures for analysis of alkyl MPAs at the low ppb level lack robustness, possibly due to differences in SPE cartridges, the

variability of urine, and inefficient derivatization in the presence of extraneous materials. Most of the more sensitive methods have been developed for use with research grade mass spectrometers, and transfer to more routine instruments may not be straightforward. A problem that occurs with the most sensitive methods is the difficulty in achieving true blanks after analyzing spiked samples.

(b) GC/MS using pentafluorobenzyl esters

Several different procedures have been reported for the trace analysis of alkyl MPAs as their pentafluorobenzyl esters. Shih *et al.*⁽⁵²⁾ isolated *i*-PrMPA from acidified (pH 1) urine or blood by SPE onto C18. The more hydrophobic PinMPA and cHexMPA were extracted onto C2. After washing with acidified sodium chloride solution, the acids were eluted with methanol. Potassium carbonate was added, the mixture concentrated to dryness, and derivatization affected by treatment with pentafluorobenzyl bromide (PFBBBr) in dichloromethane using an 18-crown-6 ether as catalyst (50 °C, 1 h). C[²H]₃-P alkyl MPAs were used as internal standards. GC/MS analysis was performed using EI, positive ion CI, and negative ion CI, limits of quantitation being 1 ng/ml for PinMPA, 5 ng/ml for cHexMPA, and 10 ng/ml for *i*-PrMPA. Fredriksson *et al.*⁽⁵³⁾ reported a similar method but extended it to include other alkyl MPAs including EMPA, and again incorporating deuterated acids as internal standards. A strong anion exchange (SAX) resin was used to capture ionized alkyl MPAs, followed by elution with 0.3 M sodium bromide solution. After concentration to dryness, derivatization was performed with PFBBBr and potassium carbonate in acetonitrile (90 °C, 1 h). The derivatized acids were further cleaned up on a Florisil cartridge. A low and variable recovery of the bis-PFB ester of MPA was noted. Analysis was by GC/MS/MS using a hybrid magnetic sector – quadrupole tandem mass spectrometer at 1000 resolution of MS1 and unit resolution of MS2. CID was performed at a high collision energy (80–100 eV) in order to produce several structurally specific product ions in addition to the major one resulting from loss of alkene from [M-PFB][–]. The method was inherently extremely sensitive, low attogram amounts being detectable in standards, but limits of detection in urine and plasma were not quoted.

Both these procedures involve a number of manipulations, including concentration to dryness before

derivatization, and a heterogeneous derivatization procedure in the presence of quite large amounts of inorganic salts. A simplified, though still quite involved procedure, uses a polymeric cartridge for extraction of acidified urine, acetonitrile for elution, and derivatization either after concentration to dryness or directly in the eluate⁽⁵⁴⁾. The derivatized solution is cleaned up on Florisil and analyzed by NICI GC/MS/MS after the method of Fredriksson. Although optimum sensitivity (~0.1 ng/ml) was achieved using an expensive hybrid magnetic sector quadrupole instrument, limits of detection in the range 0.1–0.5 ng/ml have been achieved using a relatively inexpensive benchtop ion trap MS. However, only one significant product ion, due to loss of alkene from [M – PFB][–], is obtained from the lower energy CID used in this system. Chromatograms from urine spiked at 1 ng/ml are shown in Figure 11. It should be noted that extraction efficiency is low (20–25 %) for the least hydrophobic acid EMPA, and if this were the main analyte of concern, then an alternative extraction procedure should be used.

Miki *et al.*⁽⁵⁵⁾ have reported a modification to the derivatization procedure, adapted from organophosphorus pesticide residue analysis, in which derivatization and concentration of the analytes are combined. Urine samples, with dipropyl phosphoric acid added as an internal standard, were passed through a cation exchange cartridge (Ag⁺ form) to remove chloride ions, and the pH of the eluate adjusted to 4.5. Derivatization was performed under phase-transfer conditions consisting of aqueous eluate with added phosphate buffer, PFBBBr in toluene, and a polymer-bound quaternary phosphonium phase-transfer catalyst (tri-*n*-butylmethylphosphonium bromide) (vigorously stirred, 85 °C, 90 min). The organic fraction was cleaned up on Florisil. This method avoids the need to concentrate an aqueous eluate to dryness, and results in a lower inorganic salt content in concentrates; however, it is experimentally complex. Detection limits were in the range 2.5–10 ng/ml using GC/MS/EI (SIM) and 0.06 ng/ml using GC/MS/NICI, although the chromatograms at 1 ng/ml showed a number of additional components (chromatograms would no doubt be much cleaner using MS/MS). The procedure could also be applied to deproteinated serum and diluted saliva.

times, and the highest mass ions are usually below m/z 200 and prone to interferences using SIM.

(d) GC-MS/GC-FPD using silyl esters

The most widely used procedures for environmental analysis of alkyl MPAs use conversion to trimethylsilyl (TMS) or *tert*-butyldimethylsilyl (TBDMS) esters. Both have also been applied to samples associated with the terrorist use of sarin or VX in Japan, although they are less sensitive than methods employing pentafluorobenzyl esters. Derivatization conditions for most applications typically involve treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) \pm 1 % trimethylsilyl chloride (TMSCl) (60 °C, 30 min) or *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide MTBSTFA + 1 % *tert*-butyldimethylsilyl chloride (TBDMSCl) (60 °C, 30–60 min).

Two methods adopted the approach of removing extraneous materials from urine and concentration to dryness prior to derivatization. Minami *et al.* ⁽⁴³⁾ used a three-layered cation exchange resin loaded with Ag^+ , Ba^{++} , and H^+ to remove chloride ion, sulfate ion, and pigments respectively. After adjustment of the pH of the eluant to ~ 3.7 , the solution was lyophilized before treatment with BSTFA + 10 % TMCS. LODs using GC/FPD were 25 ng/ml for *i*-PrMPA and EMPA, rather higher for MPA. Nakajima *et al.* ⁽⁴²⁾ passed urine through C18 to remove hydrophobic material and then a cation exchange resin (H^+ form) to remove metal ions. After concentration to dryness, the residue was reconstituted in tetrahydrofuran and derivatized with MTBSTFA in pyridine, NaCl added, and the derivative partitioned into hexane containing *p*-cyanophenyl dimethylphosphate as an internal standard. Using GC/FPD, the LOD for MPA was 10 ng/ml (LOD for *i*-PrMPA not quoted).

Katagi *et al.* ⁽⁵⁷⁾ detected EMPA in serum as its TBDMS derivative. Serum was deproteinized by micro-ultrafiltration and extracted with dichloromethane for unmetabolized VX [and VX metabolite (17)]. The aqueous fraction was acidified with oxalate buffer (pH 1.68), sodium chloride added to salt out the analyte, and EMPA extracted into acetonitrile. After concentration to dryness, the residue was derivatized with MTBSTFA + 1 % TBDMSCl (60 °C, 30 min). The LOD by GC/EI/MS was 3 ng/ml monitoring the most intense ion

(m/z 153), 40 ng/ml using full scan EI, and 80 ng/ml using full scan isobutane CI.

(e) LC/MS/MS

Although LC/MS has generally been less sensitive than GC/MS for alkyl MPAs ⁽⁵⁸⁾, the latest LC/MS/MS instrumentation is enabling lower LODs to be achieved. Noort *et al.* ⁽⁵⁹⁾ reported a sensitive micro-LC/MS/MS method for the detection of *i*-PrMPA in serum. $\text{C}[\text{H}]_3$ -*i*-PrMPA was added as an internal standard, the solution acidified with dilute sulfuric acid, and *i*-PrMPA extracted with isobutanol/toluene 1:1. A microbore PRP-X100 (anion exchange) column was used for LC, isocratically eluted with $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ 1:1 plus 0.5 % formic acid. The LOD was 1–4 ng/ml using either positive or negative ion ESI/MS/MS. Ions monitored were $[\text{M} - \text{H}]^- \rightarrow [\text{M} - \text{H} - \text{C}_3\text{H}_6]^-$ (m/z 137 \rightarrow 95) in negative mode and $\text{MH}^+ \rightarrow [\text{MH} - \text{C}_3\text{H}_6]^+$ (m/z 139 \rightarrow 97) in positive mode (plus the corresponding ions from the internal standard). The method was applied successfully to human samples (see below).

5.3 Detection in Cases of Human Poisoning

The only reported incidents of nerve agent poisoning, where biomedical samples have been obtained, are those resulting from terrorist dissemination of sarin in Matsumoto (1994) and the Tokyo subway (1995), plus an assassination using VX, also in Japan ⁽⁶⁰⁾. In contrast to the CW incidents involving sulfur mustard, many of the biomedical samples associated with these terrorist attacks were collected within hours of the event.

Very high levels of MPA and *i*-PrMPA were detected as their TBDMS derivatives by GC/FPD in the urine of a Matsumoto casualty rendered unconscious and with low-blood AChE activity ⁽⁴²⁾. Urine was collected over a 7-day period. Concentrations of *i*-PrMPA and MPA respectively were: day 1, 760 ng/ml and 140 ng/ml; day 3, 80 ng/ml and 20 ng/ml; day 7, 10 ng/ml, MPA not detected (no standard of *i*-PrMPA was available and quantitation was based on detector response to MPA). The exposure was estimated as 2.79 mg of sarin, making crude assumptions on the percentage that would be excreted. *i*-PrMPA was detected as the

TMS derivative by GC/FPD in urine collected over 7 days from casualties of the Tokyo attack⁽⁴³⁾. Concentrations were not reported but the estimated exposures were 0.13–0.25 mg of sarin in a comatose patient and 0.016–0.032 mg in less severely intoxicated patients. Using LC/MS/MS and a more rigorous method of quantitation, *i*-PrMPA was detected underivatized in serum at concentrations of 3–136 ng/ml in four casualties of the Matsumoto incident and 2–100 ng/ml in 13 casualties of the Tokyo attack⁽⁵⁹⁾. All samples were taken within 2.5 hours of hospitalization. High levels of *i*-PrMPA correlated with low levels of butyrylcholinesterase activity. Other positive analyses associated with these incidents were obtained by identification of inhibited cholinesterase, and are reported in Part B.

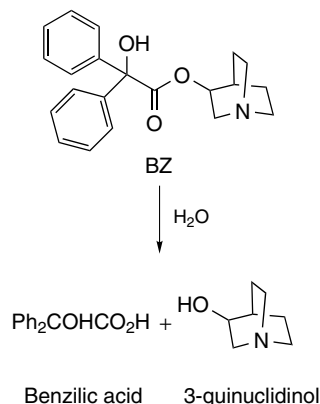
EMPA was detected as its TBDMS derivative by GC/MS and GC/MS/MS in the serum of a subject assassinated by application of VX from a disposable syringe to the neck⁽⁴⁶⁾. A concentration estimated as 1250 ng/ml was detected in serum from blood collected 1 h after the exposure and stored at -20°C for several months. 2-(Diisopropylaminoethyl) methyl sulfide (**17**) was also detected at an estimated concentration of 143 ng/ml.

6 3-QUINUCLIDINYL BENZILATE, BZ

BZ is an incapacitating agent that acts on the central nervous system, by reversible blockade of muscarinic-type receptors in the cholinergic nervous system. Incapacitating doses are in the low microgram per kilogram range. Mydriasis is one overt symptom indicating possible exposure. Unlike the vesicants and nerve agents, BZ is not known to form covalent bonds with glutathione or nucleophilic sites on macromolecules. It has been much less of a concern as a CW agent than nerve agents and vesicants, and this is reflected in the paucity of methods for the analysis of biomedical samples.

6.1 Metabolism

The metabolism of BZ appears to be dominated by simple hydrolysis to benzilic acid and 3-quinuclidinol.



6.2 Analytical Methods

BZ and 3-quinuclidinol can be analyzed underivatized by GC/MS or LC/MS, but retention times for BZ are long, sensitivity is modest, and it tends to decompose to benzophenone on hot surfaces. Byrd *et al.*^(61,62) described a GC/MS method using TMS derivatives for the determination of BZ and its hydrolysis products in urine. Each analyte was isolated from urine using separate SPE extraction procedures. BZ and benzilic acid were isolated on C18 after basification and acidification of urine respectively. Quinuclidinol was more difficult to isolate; recoveries of 40–60 % were obtained at pH 6–7 using Florisil SPE. Concentrated residues were derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) in acetonitrile (3 h, 70°C for BZ, 15 min ambient temperature for benzilic acid and 3-quinuclidinol). The method used isotope dilution, requiring ^{18}O -quinuclidinol, $^2\text{H}_5$ -benzilic acid, and $^{18}\text{O}, ^2\text{H}_5$ -BZ. Urine was treated with glucuronidase prior to analysis, although there is no information on the formation of glucuronides. Neither BZ-TMS nor benzilic acid TMS give significant M^+ ions in their EI mass spectra, but share a common base peak $[\text{Ph}_2\text{COTMS}]^+$, m/z 255 suitable for SIM. Quinuclidinol-TMS gives a moderately strong (50 %) M^+ , m/z 199 suitable for SIM. Both BZ-TMS and quinuclidinol-TMS tend to exhibit peak tailing on low polarity GC columns. The method could detect BZ at concentrations of 0.5 ng/ml and the hydrolysis products at 5 ng/ml, using large (20 ml) aliquots of urine for each analyte. Benzilic acid also forms a TBDMS derivative but

this has not been applied to biomedical sample analysis.

7 PHOSGENE

Phosgene was used as a major CW agent in WW I and was responsible for the greatest number of CW induced mortalities. It causes a lethal pulmonary oedema after an asymptomatic lag phase of up to 24 h. Its specific mechanism of action is unknown, but is assumed to involve reactions with nucleophilic sites on macromolecules.

7.1 Metabolism

Aspects of phosgene metabolism have been periodically reported, particularly as an active metabolite of chloroform. It will react with two molecules of glutathione to form a bis conjugate ⁽⁶³⁾ and with cysteine to form 2-oxothiazolidine-4-carboxylic acid ⁽⁶⁴⁾, but it does not appear to have been established if significant amounts of these or related compounds are excreted in urine. As an active metabolite of chloroform, phosgene reacts with the polar heads of phospholipids ⁽⁶⁵⁾. Its reactions with blood proteins are described in Part B.

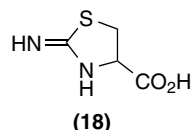
8 HYDROGEN CYANIDE

Like phosgene, hydrogen cyanide (HCN) was used in WW I, but on a smaller scale and with limited effect. It is produced in thousands of tons as a legitimate industrial chemical and fumigant. Apart from its status as a CW agent, plus its occasional use in human poisoning, HCN is a toxic component of smoke produced by cigarettes and fires. HCN is known as a lethal 'blood' gas. It reacts with the trivalent iron of cytochrome oxidase, thereby inhibiting aerobic cell metabolism and starving the blood of oxygen.

8.1 Metabolism

HCN can react with both nucleophiles and electrophiles, depending on the conditions. Most

of the cyanide in blood concentrates in the red cells bound to methemoglobin, which acts as a cyanide sink. It can be released by heating with acid. Cyanide is metabolized to thiocyanate (SCN⁻), through the action of a sulfotransferase enzyme called rhodanese, located mainly in the liver. Cyanide is excreted primarily as thiocyanate in urine. Approximately 15 % of absorbed cyanide reacts with cystine to form 2-iminothiazolidine-4-carboxylic acid (**18**). Cyanide also reacts with vitamin B12 and appears as cyanocobalamin in urine ⁽⁶⁶⁾ (and references therein).



8.2 Analytical Methods

8.2.1 Overview

Numerous methods have been reported for the analysis of cyanide and thiocyanate in biomedical samples, mostly for the determination of cyanide levels in smokers and fire victims rather than cases of deliberate poisoning. These methods include visible, ultraviolet (UV) and fluorescence based spectrometric methods, electrochemical methods using ion selective electrodes, and GC with nitrogen-phosphorus detection (NPD), electron capture detection (ECD), or MS. It is not intended to cover all of these but focus mainly on chromatographic methods.

8.2.2 US Army Method for Cyanide

The US Army Medical Research Institute of Chemical Defence employs an automated microdistillation assay for cyanide in blood, using fluorometric detection ⁽⁶⁷⁾. For assaying free CN⁻, plasma is mixed with and dialyzed against pH 7.4 phosphate buffer. For an assay of total blood cyanide, blood is mixed with saline containing Triton X (which hemolyzes the red cells), treated with 0.5 % sulfuric acid and dialyzed against 0.25 % sulfuric acid. Cyanide is

isolated by microdistillation and assayed fluorometrically. The automated procedure takes approximately 16 min.

8.2.3 GC and GC/MS Methods for Cyanide and Thiocyanate

A number of strategies have been employed for GC determination of cyanide and thiocyanate in blood. The major ones are the direct analysis of cyanide in the headspace, conversion of cyanide and thiocyanate to cyanogen chloride, and derivatization to pentafluorobenzyl cyanide and pentafluorobenzyl thiocyanate. Selected recent examples are reported below.

Methods based on headspace analysis provide low limits of detection. Typically, blood is incubated for up to ~30 minutes with phosphoric acid at temperatures between 50 °C and 70 °C. Ascorbic acid may be added to blood to prevent the conversion of thiocyanate to cyanide⁽⁶⁸⁾; acetonitrile or propionitrile may be added as internal standards. Cryogenic oven trapping of cyanide from the headspace combined with GC/NPD analysis provided a detection limit of 2 ng/ml⁽⁶⁹⁾. Alternatively, solid-phase microextraction of the headspace (using a Carbowax/divinylbenzene coated fiber) gave a detection limit of 20 ng/ml⁽⁷⁰⁾. Calafat and Stanfill⁽⁷¹⁾ reported an experimentally simple high throughput method using a fully automated headspace GC/NPD system, that provided a detection limit of 14 ng/ml. After addition of ascorbic acid, cyanide was liberated by heating blood with phosphoric acid (60 °C, 5 min). The assay was validated on samples from fire victims.

In an alternative approach, cyanide and thiocyanate can be converted to cyanogen chloride using the chlorinating agent chloramine T. Conversion can be performed in solution or in the headspace. Conversion in solution followed by headspace analysis gave a detection limit of 5 ng/ml by gas chromatography/electron capture detection (GC/ECD)⁽⁷²⁾. Conversion in the headspace above acidified blood, in a precolumn packed with chloramine T powder and attached to the injection port of the GC, gave a detection limit of 50 ng/ml⁽⁷³⁾.

Conversion to pentafluorobenzyl derivatives provides an alternative and sensitive assay for both cyanide and thiocyanate, and allows sensitive detection by ECD, or by MS using SIM at

a much higher mass than the native molecule. Chen *et al.*⁽⁷⁴⁾ reported a two-phase derivatization of several anions, including cyanide and thiocyanate, in potassium borate buffer (pH 9.5) with PFBBBr in dichloromethane, plus an immobilized phase-transfer catalyst (Kryptofix 222 B polymer). Using GC/ECD, sub-nanomolar concentrations of cyanide could be detected in urine and saliva. Kage *et al.*⁽⁷⁵⁾ analyzed cyanide and thiocyanate in blood using a two-phase pentafluorobenzylation procedure with PFBBBr and tetradecyldimethylbenzylammonium chloride as phase-transfer agent. For cyanide determination, blood was pretreated with sodium sulfite to prevent oxidation of blood cyanide, and with trichloroacetic acid to precipitate proteins. Thiocyanate in blood was determined without deproteinization. GC/ECD was used for quantitative analysis and GC/MS for qualitative analysis. Detection limits for cyanide and thiocyanate using ECD were 0.01 and 0.003 $\mu\text{mol/ml}$ blood respectively. The EI mass spectrum of pentafluorobenzyl cyanide gave a molecular ion (m/z 207) as the base peak; pentafluorobenzylthiocyanate gave a weak M^+ and a base peak $[M - \text{SCN}]^+$ at m/z 181.

8.2.4 Iminothiazolidine Carboxylic Acid

Lundquist *et al.*⁽⁷⁶⁾ reported a method for the analysis of the metabolite 2-aminothiazoline-4-carboxylic acid (**18**) in urine using LC with fluorescence detection after conversion to *N*-carbamylcysteine by heating with alkali. The analyte was concentrated from urine by cation exchange resin and further processed to remove interfering thiols and disulfides. The LOD was rather high at 0.3 μM .

8.3 Application to Human Casualties

Cyanide and thiocyanate are normal constituents of blood⁽⁷⁷⁾. Sources of cyanide include some foods, for example, cyanogenic glycosides in bitter almonds, fruit seeds, and a number of plants, cigarette smoke, and smoke from fires. The blood concentration of cyanide in healthy subjects was 8.4 ± 3.1 ng/ml by the method of Ishii *et al.*⁽⁶⁹⁾; in nine fire victims concentrations determined were 687 ± 597 ng/ml. In smokers, cyanide levels in blood may rise to ~500 ng/ml.

Kage *et al.* ⁽⁷⁵⁾ applied their GC/ECD/GC/MS method to the analysis of blood levels in two casualties who died from cyanide poisoning. Blood levels of cyanide and thiocyanate in a subject who died following ingestion of sodium cyanide were 0.52 and 0.10 $\mu\text{mol/ml}$ respectively (≈ 13 and $5.6 \mu\text{g/ml}$). Levels determined in a fatal victim of smoke inhalation were 0.28 and 0.13 μmol respectively (≈ 7.3 and $7.5 \mu\text{g/ml}$). Fatal levels of cyanide are estimated as ~ 0.05 – $0.1 \mu\text{mol/ml}$ (≈ 1.3 – $2.6 \mu\text{g/ml}$). The lower levels of thiocyanate in comparison to cyanide in the blood were attributed to the sudden death; they were twice the mean levels ($\sim 0.06 \mu\text{mol/ml}$) found in cigarette smokers. 2-Aminothiazoline-4-carboxylic acid was detected in the urine of moderate cigarette smokers at concentrations between <0.3 – $1.1 \mu\text{M}$ ⁽⁷⁶⁾.

9 CONCLUSIONS

Free metabolites that can be used as unequivocal biological markers of human exposure have been identified for sulfur mustard and nerve agents, and sensitive analytical methods developed for their detection. These have been validated by the analysis of samples from casualties of poisoning by sulfur mustard, sarin, and VX. Putative metabolites have been identified in animal studies for nitrogen mustards and lewisite. Phosgene and cyanide have received little attention with regard to retrospective identification of exposure and significant background levels of metabolites in control subjects may be a complicating factor.

Substantial progress has been made in this research area over the past two decades. New metabolites continue to be identified and new analytical methods developed. Many of the analytical methods have been developed primarily for retrospective identification of exposure in cases of allegations of CW use. In this scenario, low limits of detection are much more important than high throughput. With the current concern for terrorist use of CW agents, new methods of analysis are being developed with greater emphasis on high throughput screening of casualties rather than low or sub-ppb limits of detection. New generations of mass spectrometers will undoubtedly lead to lower limits of detection, particularly in

LC/tandem MS. However, a major challenge is the development of sensitive methods that can be performed on the more routine instruments found in most analytical laboratories rather than the very expensive instruments used for research. Another factor that requires consideration is the availability of analytical standards. The most encouraging aspect is that, in combination with the analysis of covalent adducts (see following chapter), there is now a high probability of confirming cases of human exposure to CW agents provided that appropriate samples are collected.

NOTE

A number of modifications/improvements to methods for the analysis of metabolites of sulfur and nitrogen mustards, and hydrolysis products of nerve agents, have been reported in a special issue of the *Journal of Analytical Toxicology*, **28** (5) (2004) pp. 305–392.

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ABBREVIATIONS AND ACRONYMS

AED	Atomic Emission Detection
BAL	British Anti-Lewisite
BSTFA	Bis(Trimethylsilyl)Trifluoroacetamide
BZ	Quinuclidinyl Benzilate
CI	Chemical Ionization
CID	Collision Induced Dissociation
CVAA	2-Chlorovinylarsonous Acid
CW	Chemical Warfare
CWC	Chemical Weapons Convention
ECD	Electron Capture Detection
EI	Electron Impact
EI/MS	Electron Impact/Mass Spectrometry

EMPA	Ethyl Methylphosphonic Acid	SPE	Solid-Phase Extraction
ESI	Electrospray Ionization	SPME	Solid-Phase Microextraction
GA	Tabun	TBDMS	Tert-Butyldimethylsilyl
GB	Sarin	TDG	Thiodiglycol
GC	Gas Chromatography	TDGO	Thiodiglycol Sulphoxide
GC/ECD	Gas Chromatography/Electron Capture Detection	TMCS	Trimethylchlorosilane
GC/FPD	Gas Chromatography/Flame Photometric Detection	TMS	Trimethylsilyl
GC/MS	Gas Chromatography/Mass Spectrometry	UV	Ultraviolet
GC/NPD	Gas Chromatog- raphy/Nitrogen–Phosphorus Detection	VX	O-Ethyl S-2-Diisopropylaminoethyl Methylphosphonothiolate
GD	Soman		
GF	Cyclohexylsarin		
HCN	Hydrogen Cyanide		
HFB	Heptafluorobutyl		
HFBI	Heptafluorobutylimidazole		
LC	Liquid Chromatography		
LC/ESI/MS/MS	Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry		
LC/MS	Liquid Chromatography/Mass Spectrometry		
LOD	Limit of Detection		
MPA	Methylphosphonic Acid		
MRM	Multiple Reaction Monitoring		
MS	Mass Spectrometry		
MS/MS	Tandem Mass Spectrometry		
MSTFA	<i>N</i> -Methyl- <i>N</i> -Trimethylsilyl- Trifluoroacetamide		
MTBSTFA	<i>N</i> -Methyl- <i>N</i> -(Tert.- Butyldimethylsilyl)Trifluoro- acetamide		
NICI	Negative Ion Chemical Ionization		
NICI/MS	Negative Ion Chemical Ionization/Mass Spectrometry		
NPD	Nitrogen–Phosphorus Detection		
OPCW	Organization for the Prohibition of Chemical Weapons		
PDMS	Poly(Dimethylsiloxane)		
PS-DVB	Polystyrene-divinylbenzene		
SAX	Strong Anion Exchange		
SCN	Thiocyanate		
SIM	Selected Ion Monitoring		

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CHAPTER 17

Methods for Retrospective Detection of Exposure to Toxic Scheduled Chemicals. Part B: Mass Spectrometric and Immunochemical Analysis of Covalent Adducts to Proteins and DNA

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1 INTRODUCTION

1.1 General

The role of biomedical sample analysis in support of investigations of CW use, and for diagnosis and monitoring of possible exposure to CW agents, has been described in the **Chapter 16** and in a recent review ⁽¹⁾. Part A of this two-part overview of biomedical sample analysis reviews the identification and analysis of free metabolites, in urine and blood, as biological indicators of

exposure to CW agents. The major disadvantage of free metabolites as biological indicators is their relatively rapid elimination from the body. Experience has shown that only ultra trace levels are likely to be detected if urine is collected >~2 weeks after the exposure. Depending on the scenario and application, it is clearly advantageous to have biological indicators that are more slowly eliminated, ideally allowing retrospective identification up to several months following an exposure. Covalent adducts with macromolecules, such as proteins and DNA, offer potentially

much longer-lived biological indicators of exposure in comparison to free metabolites. This chapter presents an overview of adducts that have been identified for CW agents, and the methods currently available for their detection. Applications to cases of human exposure are reported.

1.2 DNA and Protein Adducts

1.2.1 DNA Adducts

Vesicants, nerve agents, and phosgene are reactive electrophiles that react covalently with nucleophilic sites on macromolecules. Reactive nucleophilic sites exist on the bases and phosphate groups of DNA molecules. An advantage of DNA as a substrate is that it is present in all tissues of the body. A disadvantage is that repair mechanisms tend to excise the alkylated moiety, resulting in a much shorter lifetime compared to alkylated proteins (for a recent review of mass spectrometry for quantitation of DNA adducts, see Koc and Swenberg⁽²⁾).

1.2.2 Protein Adducts

Several of the constituent amino acids of proteins contain nucleophilic groups; examples are cysteine (SH), serine, tyrosine (OH), lysine (NH₂), and aspartic and glutamic acids (CO₂H). Covalent adducts with proteins offer potentially long-lived biological indicators of exposure, provided they survive the normal lifetime of the macromolecule. Particularly useful as biological indicators are adducts with the abundant blood proteins hemoglobin and albumin (for recent reviews, see Törnqvist *et al.*⁽³⁾ and Boogaard⁽⁴⁾). These two proteins normally interact by purely chemical reactions of peripheral amino acid residues with the electrophile. Measurement of hemoglobin adducts is a well-established method for biomonitoring of environmental or occupational exposure to alkylating carcinogens^(3,4). Hemoglobin adducts are generally stable (in contrast to alkylated DNA) and most appear to have lifetimes in humans similar to the native protein (approximately 120 days). Consequently, the adducts may be detectable for a long period after the actual exposure. In case of chronic exposures, the adducts will accumulate in time. Hemoglobin is abundant

in human blood (140 mg/ml) and can be readily isolated as globin. Albumin is also a very abundant protein present in blood (30–45 mg/ml) but has a shorter lifetime than hemoglobin (half-life in humans 20 days)⁽³⁾. In the case of nerve agents, a selective catalytic reaction with two less abundant proteins, acetyl- and butyrylcholinesterase, provides very specific biological indicators of exposure.

1.3 Analytical Methods

The analysis of adducts with macromolecules can be more demanding than the analysis of free metabolites. In the case of noncatalyzed adduct formation, only a small fraction of the available macromolecule reacts with the CW agent, and the adduct must therefore be detected against a heavy background of native macromolecule. DNA adducts can be detected by mass spectrometric assays after enzymatic or chemical hydrolysis to alkylated nucleotides, nucleosides, or bases⁽²⁾. Other techniques, such as ³²P-postlabeling and immunoassays, are also applicable but require mass spectrometric confirmation in the case of forensic samples. A number of different strategies have been applied to the mass spectrometric analysis of protein adducts. One approach is a nonselective enzymatic or acidic digestion of the protein to the individual amino acids, with detection of the discrete amino acid adduct. This approach is advantageous in certain cases but the chemical background produced can be very high. An exception is the selective cleavage of alkylated N-terminal valine on hemoglobin (see Section 2.2.2). Alternatively, a more selective enzymatic digestion can be used, for example, with trypsin, to provide a small alkylated or phosphorylated peptide. Both of these methods may require demanding sample preparation procedures, usually combined with LC/electrospray tandem MS (LC/MS/MS) analysis. GC/MS/MS is applicable to some amino acid adducts. Both approaches may be complicated by steric hindrance of the digestive enzyme by the bound agent residue, for example, in some cases, the enzyme Pronase may not efficiently digest the protein to its individual amino acids. A third approach involves chemical displacement of the covalently bound CW agent residue. This provides the original CW agent, a related compound, or its hydrolysis product, which is analyzed in

the same manner as a free metabolite. The advantage of this approach is that sample preparation may be simpler, and analysis performed using gas chromatography/mass spectrometry (GC/MS) or GC/MS/MS. Immunoassays provide complementary methodology for screening and monitoring purposes.

2 SULFUR MUSTARD

Sulfur mustard (mustard gas) remains one of the CW agents of greatest concern because of its ease of production, favorable physicochemical properties, and potent vesicant action. It is a bifunctional alkylating agent, which reacts rapidly under physiological conditions with nucleophilic sites in proteins and DNA to form covalent adducts, via an intermediate episulfonium ion (see Figure 1). In the sections below, the various adducts (as unambiguously elucidated in recent years by mass spectrometry) are addressed, and methods for their analysis are discussed.

2.1 Sulfur Mustard Adducts with DNA

2.1.1 Sites of Reaction

By analogy to several nitrogen mustard-based antitumor agents, the primary site of DNA alkylation by sulfur mustard is the N7 position of deoxyguanosine residues^(5,6). Upon depurination of the resulting N7-(2-hydroxyethylthioethyl)-2'-deoxyguanosine, N7-(2-hydroxyethylthioethyl)guanine (N7-HETE-Gua) is obtained (see Figure 2). Minor amounts of the N7-guanine di-adduct and the N3-adenine adduct were also detected.

2.1.2 Analytical Methods

(a) Mass spectrometric methods

GC/MS analysis of (N7-HETE-Gua) proved problematic. Derivatization with heptafluorobutyric acid anhydride and pentafluorobenzyl bromide was troublesome and silylation afforded a derivative with poor gas chromatographic properties. The underivatized compound could however be conveniently

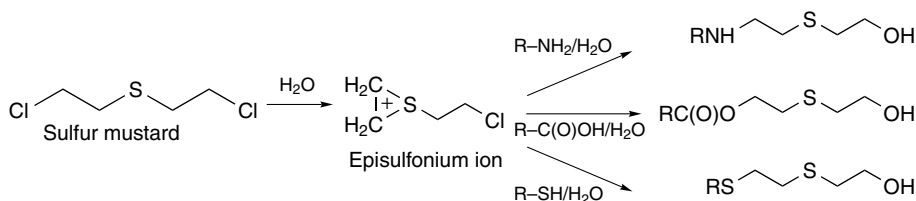


Figure 1. Sulfur mustard adduct formation with various nucleophiles

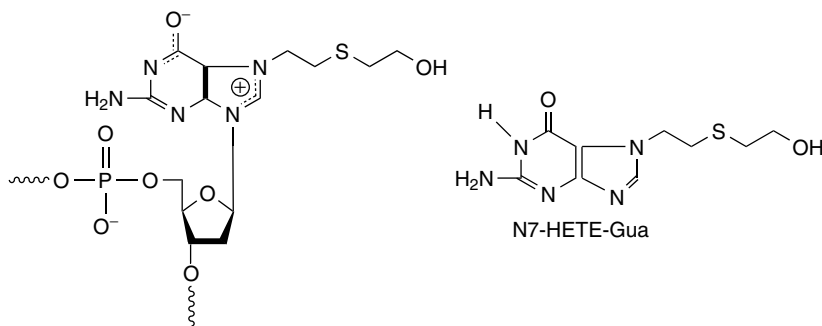


Figure 2. N7-Deoxyguanosine adduct of sulfur mustard and derived N7-(2-hydroxyethylthioethyl)guanine (N7-HETE-Gua). (Reprinted from Toxicology and Applied Pharmacology, Vol. 184, D. Noort, H.P. Benschop and R.M. Black, Biomonitoring of Exposure to Chemical Warfare Agents: A Review, pages 116–126 (2002), with permission from Elsevier Science.)

analyzed in urine using LC/MS/MS, using a C18 cartridge for extraction ⁽⁷⁾. The adduct was readily detected in the urine of guinea pigs exposed to sulfur mustard, although levels dropped rapidly after 36–48 h, and after processing of skin and blood samples of animals exposed to sulfur mustard. Using similar methodology, N7-HETE-Gua was detected in the spleen and liver of rats exposed percutaneously to sulfur mustard ⁽⁸⁾.

(b) Other physicochemical methods

The group of Ludlum developed a high-performance liquid chromatography (HPLC) method for the analysis of N7-HETE-Gua ⁽⁹⁾ and also a ³²P-postlabeling method for N7-HETE-deoxyguanosine 5'-phosphate ⁽¹⁰⁾.

(c) Immunoassay method

An enzyme-linked immunosorbent assay (ELISA) was developed by Van der Schans and colleagues for detection of the mustard adduct within DNA, using monoclonal antibodies raised against N7-HETE-guanosine-5'-phosphate coupled to keyhole limpet hemocyanin ⁽¹¹⁾. The ELISA was successfully applied in toxicokinetic studies in which levels of adducted DNA were followed in conjunction with measurement of intact sulfur mustard ⁽¹²⁾.

2.1.3 Application to Human Casualties

The ELISA for detection of the DNA adduct was successfully applied to blood samples from two casualties of the Iraq–Iran conflict. These samples were collected 22 and 26 days following the alleged exposure to sulfur mustard ⁽¹³⁾. Concentrations found in lymphocytes and granulocytes were equivalent to similar levels found in human blood after treatment *in vitro* with 0.015–0.43 μ M sulfur mustard.

2.2 Sulfur Mustard Adducts with Hemoglobin

2.2.1 Sites of Reaction

Distribution studies of ³⁵S-sulfur mustard in rats after cutaneous exposure to sulfur mustard, and human blood treated *in vitro*, showed that a small percentage of radioactivity remained associated with the hemoglobin and persisted for the lifetime of the

red cells ⁽¹⁴⁾. The alkylation of proteins by sulfur mustard was studied in the 1940s and 1950s by various groups (for a review, see Wheeler ⁽¹⁵⁾), indicating that alkylation of, for example, carboxyl, amino, and sulfhydryl groups, readily occurs. Definitive evidence for specific alkylation sites has been obtained more recently by using modern mass spectrometric techniques. LC/MS/MS analysis of tryptic digests, of hemoglobin treated with radio-labeled sulfur mustard, identified alkylation on 6 different histidine residues, 3 glutamic acid residues, and both of the N-terminal valines ^(16,17); most of these residues are peripherally located, as should be expected. Alkylated cysteine, aspartic acid, lysine, and tryptophan were also detected in Pronase digests. The N1 and N3 histidine adducts were found to be the most abundant adducts.

2.2.2 Analytical Methods

Although the degree of alkylation of the N-terminal valine in human hemoglobin is only 1–2 % of the total alkylation induced in hemoglobin upon treatment of human blood with sulfur mustard, N-alkylated N-terminal valine has the advantage as a biomarker of exposure, that it can be selectively cleaved from hemoglobin by a modified Edman procedure. This method, using pentafluorophenyl isothiocyanate as reagent, was originally reported by Törnqvist *et al.* ⁽¹⁸⁾ (see Figure 3) for other alkylating agents. Globin is isolated from human blood using standard procedures, dissolved in formamide, and treated with the reagent at 60 °C for 2 h. The derivatized N-alkylated valine is extracted into diethyl ether, the solvent removed and the residue redissolved in toluene. Analysis of the resultant pentafluorophenyl thiohydantoin, using negative ion GC/MS/MS after further derivatization with heptafluorobutyric anhydride or heptafluorobutyrylimidazole, provided a sensitive method for the detection of the N-alkylated valine ^(19,20). The lowest detectable exposure level of human blood *in vitro* was 0.1 μ M. An *in vivo* study with marmosets demonstrated the potential for hemoglobin adducts as relatively long-lived biological markers of sulfur mustard poisoning ⁽²¹⁾. After administration of a single dose of sulfur mustard (4.1 mg/kg), the N-terminal valine adduct was still detectable after 94 days (see Figure 4). Remarkably, the adduct

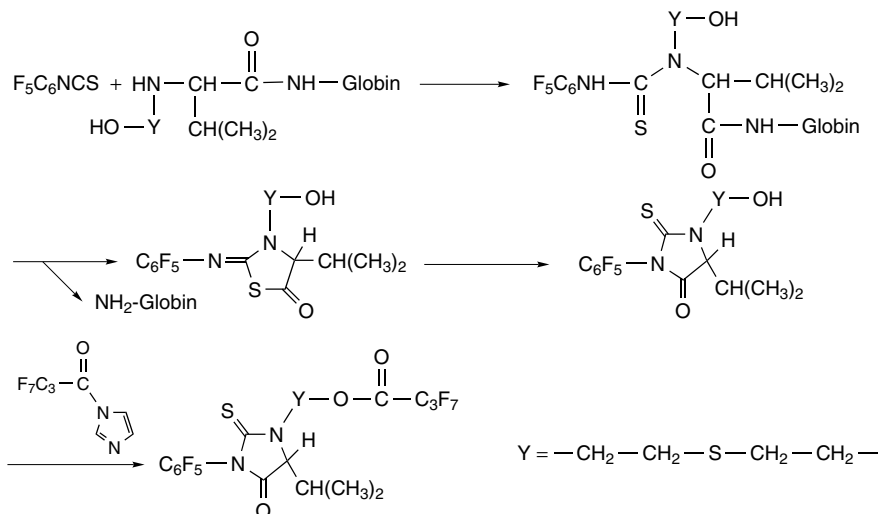


Figure 3. Modified Edman degradation procedure of globin alkylated by sulfur mustard. (Reprinted from Toxicology and Applied Pharmacology, Vol. 184, D. Noort, H.P. Benschoop and R.M. Black, Biomonitoring of Exposure to Chemical Warfare Agents: A Review, pages 116–126 (2002), with permission from Elsevier Science.)

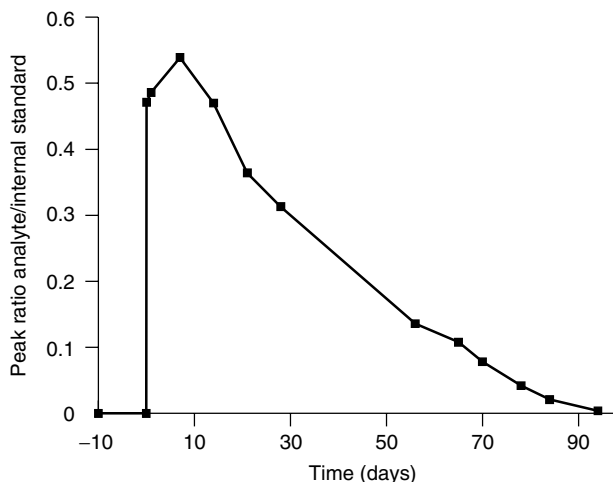


Figure 4. Persistence of sulfur mustard adduct to N-terminal valine residue of hemoglobin in blood of a marmoset after sulfur mustard administration (4.1 mg/kg, i.v.) at $t = 0$. At the time points indicated blood samples were collected, globin was isolated and analyzed by using the modified Edman degradation for determination of the N-terminal valine adduct. Globin from human blood exposed to d_8 -sulfur mustard (10 μM) was used as an internal standard. (Reprinted from Toxicology and Applied Pharmacology, Vol. 184, D. Noort, H.P. Benschoop and R.M. Black, Biomonitoring of Exposure to Chemical Warfare Agents: A Review, pages 116–126 (2002), with permission from Elsevier Science.)

level increased for several days after administration of sulfur mustard, which indicates a pronounced stability of the intact agent.

The histidine adduct is the most abundant adduct formed after exposure of hemoglobin to sulfur

mustard, and is stable upon hydrolysis of globin in 6 N HCl. However, its GC/MS analysis is hampered by the high polarity and poor thermal stability of volatile histidine derivatives. A sensitive method was developed using LC/MS/MS after

derivatization to the N-9-fluorenylmethoxycarbonyl derivative ⁽²²⁾. Globin was hydrolyzed in 6N HCl (110 °C, 24 h), the analyte concentrated by extraction on a PRS cation exchange cartridge and, after concentration to dryness, derivatized with 9-fluorenylmethoxycarbonyl chloride. This procedure is rather laborious and proved to be less sensitive than the modified Edman degradation.

Recently, matrix-assisted laser desorption ionization/time-of-flight/mass spectrometry (MALDI/TOF/MS) of intact adducted hemoglobin was explored as a diagnostic tool for the confirmation of exposure to sulfur mustard ⁽²³⁾. Multiple alkylated species were observed from incubates of hemoglobin with sulfur mustard; however, the methodology has not yet been reported for diagnostic purposes.

2.2.3 Application to Human Casualties

Using the modified Edman procedure, the N-terminal valine adduct has been detected in several cases of human exposure to sulfur mustard. The long lifetime of hemoglobin adducts was demonstrated in the case of blood samples from two Iranian CW casualties taken 22–26 days after alleged exposure ⁽¹³⁾. One victim suffered from skin injuries compatible with sulfur mustard intoxication but had no other injuries; the symptoms of the other victim were only vaguely compatible with sulfur mustard intoxication. The N-terminal valine adduct levels corresponded with those found in human blood after treatment *in vitro* with approximately 0.9 μ M sulfur mustard. The results were confirmed by immunochemical analysis of DNA adducts in lymphocytes from the same blood samples (*vide supra*). Positive results were also reported for blood samples from four Iranian casualties, collected between 5 and 10 days following the alleged CW attack ⁽²⁰⁾. Concentrations

were low, equivalent to 0.3–0.8 ng/ml of the valine adduct. A similar low concentration was found in the blood of a casualty accidentally exposed to sulfur mustard (blood collected 2–3 days after the exposure). Alkylated histidine was detected in blood from the same set of four Iranian casualties, and the accidental casualty. Concentrations were equivalent to 0.7–2.5 ng/ml ⁽²⁰⁾. It should be noted that in all of the examples above, the concentrations of adduct detected were low in relation to the limits of detection, and scrupulous controls must be run during the analyses.

2.3 Sulfur Mustard Adducts with Albumin

2.3.1 Site of Reaction

Sulfur mustard was shown by Noort *et al.* ⁽²⁴⁾ to alkylate the cysteine-34 residue in human serum albumin. The site of alkylation was identified in a tryptic digest of albumin from blood exposed to [¹⁴C] sulfur mustard. The cysteine-34 residue is the only free cysteine residue in human serum albumin and has a relatively low pKa, caused by intramolecular stabilization of the thiolate anion. It has previously been identified as a nucleophilic site capable of reacting with various electrophiles ^(25,26).

2.3.2 Analytical Method

A sensitive method for analysis of the sulfur mustard adduct to this residue was developed on the basis of Pronase digestion of alkylated albumin to the tripeptide S-[2-[(hydroxyethyl)thio]ethyl]-Cys-Pro-Phe, and detection using micro-LC/tandem MS ⁽²⁴⁾ (see Figure 5). Albumin was isolated from

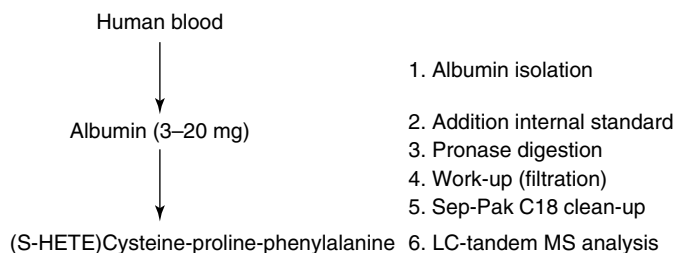


Figure 5. Procedure for analysis of sulfur mustard adduct to cysteine-34 residue in human serum albumin

blood, digested with Pronase, and the alkylated tripeptide concentrated by extraction on a C18 cartridge. The detection limit for *in vitro* exposure of human blood to sulfur mustard was determined to be 1 nM (see Figure 6), that is, two orders of magnitude more sensitive than the modified Edman degradation. An additional advantage of this assay is that albumin can be isolated very rapidly from plasma by means of affinity chromatography⁽²⁷⁾. Compared to the assay for analysis of N-terminal valine adduct (*vide supra*), it can be expected that this assay is less retrospective, due to the faster elimination rate of albumin adducts (half-life of albumin of 20–25 days versus the life span of hemoglobin of 120 days).

Recently, it was shown that various nitrogen mustard-based cytostatics, for example, melphalan and cyclophosphamide, reacted with the cysteine 34 residue of human serum albumin in an analogous way. The tripeptide assay could be applied to samples of cancer patients treated with these cytostatics⁽²⁸⁾, which holds promise for optimization of chemotherapy with these agents by intensive screening of adduct levels in patients.

2.3.3 Application to Human Casualties

The analytical procedure for S-[2-[(hydroxyethyl)thio]ethyl-Cys-Pro-Phe was successfully applied to blood samples from nine Iranian casualties of the Iraq–Iran war, all exhibiting skin injuries compatible with exposure to sulfur mustard. The blood samples were collected 8–9 days after the alleged exposure and stored at -70°C . The albumin adduct was detected in all cases, at levels estimated as corresponding to those after *in vitro* exposure of human blood to mustard concentrations ranging from 0.4–1.8 μM .

2.4 Adducts with Aspartic and Glutamic Acid Residues

2.4.1 Sites of Reaction

Korte *et al.*⁽²⁹⁾ have recently reported an alternative approach that determines thiodiglycol released by hydrolysis from blood proteins. Both globin and albumin contain numerous aspartic and glutamic

acid residues, some of which have been shown to be esterified by sulfur mustard (see Section 2.2.1)⁽¹⁷⁾.

2.4.2 Analytical Method

Blood proteins were precipitated and esterified aspartic and glutamic acid residues hydrolyzed with sodium hydroxide (1M NaOH, 2 h, 70°C). After neutralization, thiodiglycol was extracted from the aqueous layer with ethyl or propyl acetate and analyzed as its bis(pentafluorobenzoyl) derivative by GC/MS with negative ion chemical ionization (as described for thiodiglycol in urine or blood in the previous chapter). The derivative was partly cleaned up by passage through a silica cartridge. The method is very sensitive and could detect thiodiglycol released from swine blood after *in vitro* exposure to 2 nM sulfur mustard, and from monkey blood at least up to 45 days following an intravenous dose of 1 mg/kg. Only a small amount of background interference was observed, equivalent to 0.5 pg thiodiglycol per mg protein.

2.5 Adducts with Keratin

2.5.1 Sites of Reaction

The use of sulfur mustard as a vesicant CW agent implies that proteins of the skin are a primary target. It was found that upon exposure of human callus to [^{14}C]sulfur mustard, a significant part of the radioactivity was covalently bound to keratin⁽³⁰⁾. Most of the radioactivity (80 %) bound to keratin could be removed by treatment with alkali, indicating the presence of adducts to glutamic and/or aspartic acid residues.

2.5.2 Analytical Method

A direct detection method was recently developed for these adducts in stratum corneum of human skin based on immunofluorescence microscopy⁽³⁰⁾. Three partial sequences of keratins containing glutamine or asparagine, adducted with a 2-hydroxyethylthioethyl group at the omega-amide function, were synthesized and used as antigens for raising antibodies. After immunization, monoclonal antibodies were obtained with affinity for keratin isolated from human callus exposed to 50 μM sulfur mustard (see Plate 1). In contrast to the immunochemical

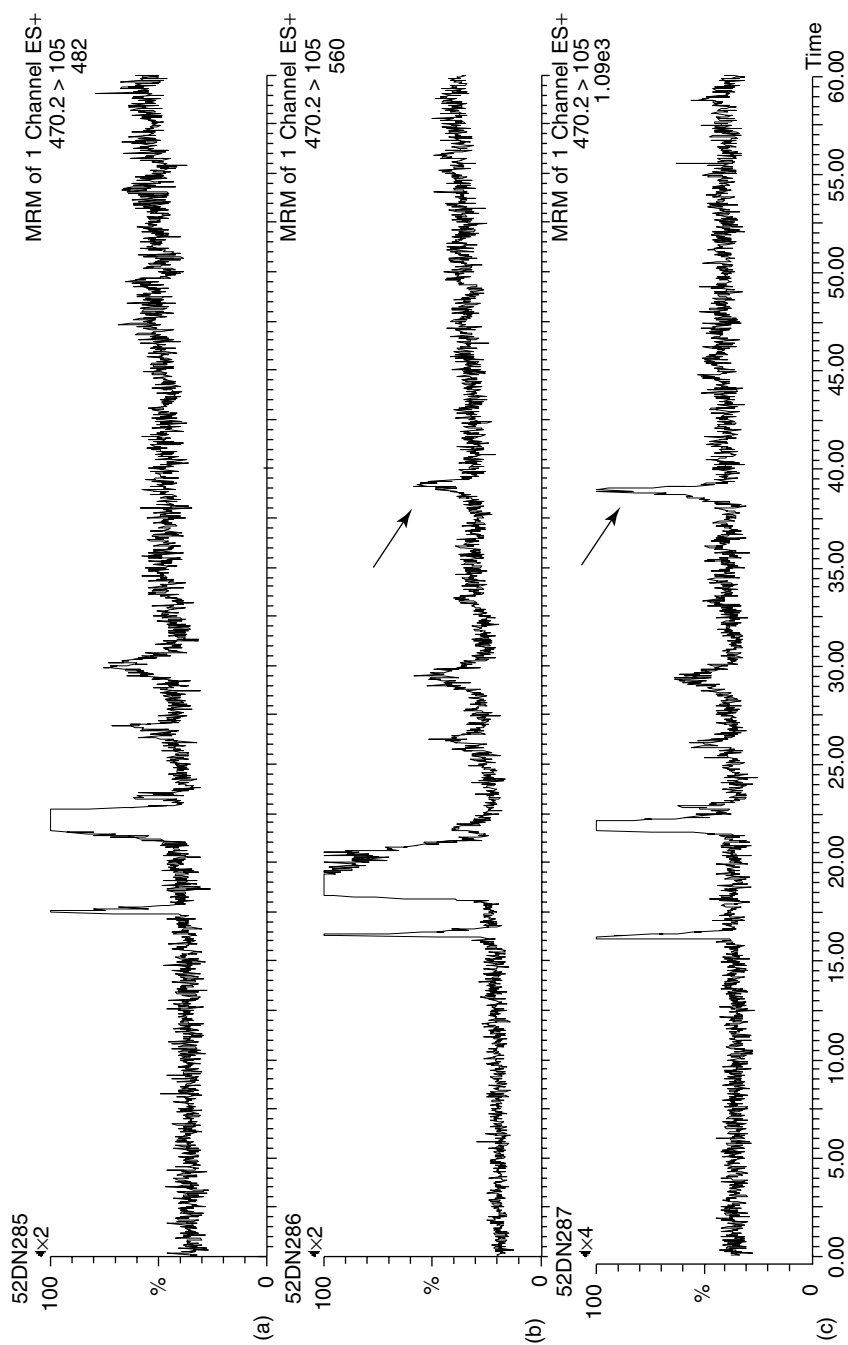


Figure 6. Trace level LC/electrospray tandem MS analysis of (S-HETE)/Cys-Pro-Phe in pronase digest of albumin (20mg) after purification on Sep-Pak C18, measuring the transition m/z 470 (MH^{+}) \rightarrow 105. Albumin was isolated from nonexposed blood (A) or from human blood that was exposed to 1 nM (B). Panel C represents the 1 nM digest after spiking with synthetic (S-HETE)/Cys-Pro-Phe. The arrow indicates the peak for (S-HETE)/Cys-Pro-Phe

method for analysis of DNA-sulfur mustard adducts, which involves laborious work-up procedures, this approach opens the way for development of a rapid detection kit that can be applied directly to the skin.

3 LEWISITE

Lewisite is the most important of the organo-arsenical CW agents. Exposure to lewisite is quite painful, and onset of symptoms occurs rapidly (seconds to minutes) ⁽³¹⁾ in contrast to sulfur mustard for which a latency period occurs of several hours between exposure and symptoms ⁽³²⁾. Although it is not known to have been used as a CW agent, lewisite is still considered a potential threat due to the relative ease of production and its rapid onset of action. Moreover, substantial stockpiles of lewisite are present in the United States, Russia, and in China abandoned by the Japanese Imperial Army. This may constitute a potential hazard for public health ⁽³³⁾. The toxicity of lewisite is *inter alia* caused by the high affinity for the vicinal di-thiol system present in dihydrolipoic acid, a component of the pyruvate dehydrogenase complex, as is the case for other arsenicals ⁽³⁴⁾. This prevents the formation of acetyl coenzyme A from pyruvate.

The most generally applied method for determination of an arsenical is by atomic absorption spectrometry (AAS) after reduction of the compound to AsH₃. However, this only provides an indication of the presence of the element as against a natural background. Lewisite rapidly hydrolyzes to 2-chlorovinylarsonous acid (CVAA; see Figure 7) in an aqueous environment such as blood plasma, and analytical methods have focused mainly on the determination of CVAA (see **Chapter 16**).

3.1 Hemoglobin Adduct

3.1.1 Site of Reaction

In view of the high affinity of arsenic for thiol functions, it can be expected that lewisite and CVAA will bind to cysteine residues of proteins. When human blood was incubated with 20 nM to 0.2 mM of [¹⁴C]lewisite, 25–50 % of the dose became associated with globin ⁽³⁵⁾. Electrospray tandem MS provided evidence for the presence of a CVAA-crosslink between the cysteine-93 and cysteine-112 residues in β -globin. Whether this was the only type of adduct has not yet been completely elucidated. It must be remarked, however, that this result was in contrast with results obtained by others for the analogous phenyldichloroarsine, for which binding to human hemoglobin could not be observed.

3.1.2 Analytical Method

CVAA could be readily displaced from globin (or whole blood) as the CVAA-BAL derivative by addition of 2,3-dimercaptopropanol (British Anti-Lewisite, BAL) (see Figure 7). The latter forms a thermodynamically favored dithiaarsenoline derivative at ambient temperature that can be extracted onto a C18 cartridge. This derivative could be analyzed very sensitively by GC/MS under electron impact conditions, after additional derivatization with heptafluorobutyrylimidazole. The lowest detectable concentration of Lewisite for *in vitro* exposure of human blood was determined to be 1 nM. *In vivo*, experiments were performed with guinea pigs (0.25 mg/kg; s.c.). The amount of CVAA-BAL isolated from blood samples clearly decreased with increasing time after exposure, as should be expected. In the blood sample taken

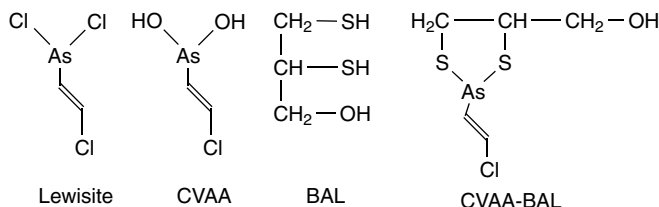


Figure 7. Chemical structures of 2-chlorovinylldichloroarsine (lewisite; L1), 2-chlorovinylarsonous acid (CVAA), British Anti-Lewisite (BAL) and the CVAA-BAL complex

10 days after exposure, the amount of isolated CVAA-BAL had decreased to 10 % of the amount at one day after exposure ⁽³⁵⁾.

3.1.3 Application to Human Casualties

No examples of biomedical sample analysis following human exposure to lewisite have been reported.

4 NERVE AGENTS

Nerve agents are the most potent of the weaponized CW agents, causing lethality at doses in the $\mu\text{g/kg}$ range. Their use in the last two decades, in the Iraq–Iran conflict, against Kurdish communities in Iraq, and by terrorists in Japan, has stimulated the development of a number of forensic methods for confirmation of exposure.

4.1 Adducts with Acetyl- and Butyrylcholinesterase

4.1.1 Site of Reaction

The extremely high toxicity of nerve agents (for a review see Black and Harrison ⁽³⁶⁾) can be attributed to the excessive cholinergic stimulation caused by inhibition of acetylcholinesterase (AChE) at neuromuscular junctions and in the central nervous system (CNS). Nerve agents react rapidly with a serine hydroxyl group in the active site of AChE, with the formation of a phosphate or phosphonate ester. The phosphorylated enzyme regenerates extremely slowly, rendering the enzyme inaccessible for its parent substrate acetylcholine. In the case of G-agents, the intact agent is present in the organism for only several hours. Therefore, intact agents are not considered good targets for retrospective detection of exposure. Interestingly, the chirality around the phosphorus atom has large implications for the toxicity of these agents. The P(–) isomers of G-agents bind to AChE much more rapidly than the P(+) isomers, which are rapidly hydrolyzed by phosphorophosphatases. Consequently, the P(–) isomers are much more toxic than the P(+) isomers ⁽³⁷⁾. In addition to binding to AChE, sequestration with the closely related plasma protein butyrylcholinesterase

(BuChE) and to carboxylesterase (CaE) occurs. After binding to AChE or BuChE, some G-agents undergo a rapid secondary reaction in which one of the substituents of the phosphyl moiety is split off. This process is called aging. Thus, in the case of inhibition by soman, the PO-pinacolyl bond is cleaved within minutes (see Ordentlich *et al.* ⁽³⁸⁾ for a detailed study), and with tabun, the P–N bond is cleaved ^(39,40). In both cases, a negatively charged phosphyl moiety results, which is resistant to reactivation by nucleophiles such as therapeutically applied oximes.

4.1.2 Analytical Methods

(a) Determination of inhibited enzyme

The oldest method to establish exposure to nerve agents is the measurement of the decrease in AChE activity in blood (see Nigg and Knaak ⁽⁴¹⁾ for a review on biomonitoring of organophosphate exposure). The original colorimetric Ellman procedure ⁽³⁹⁾, or modified variations thereof ⁽⁴²⁾, are generally used for occupational health screening and therapeutic monitoring of pesticide-poisoned patients. Although the method is rapid and applicable under field conditions, it suffers from serious drawbacks. Firstly, it does not identify the organophosphate. Secondly, the specificity of the method is low, that is, various unrelated chemicals (e.g. carbamate pesticides) can contribute to inhibition of AChE. Thirdly, it does not provide reliable evidence for organophosphate exposure at inhibition levels less than 20 %, which is due to both substantial intraindividual and interindividual variations, while control activity levels are often not available ⁽⁴³⁾. Finally, it is less suitable for retrospective detection of exposure due to *de novo* synthesis of enzyme. Nevertheless, measurement of AChE inhibition is still the most widely used method for assessment of exposure to nerve agents.

(b) Fluoride reactivation of phosphorylated binding sites

In principle, organophosphate-inhibited BuChE in human plasma is a persistent (half-life 5–16 days) and relatively abundant (plasma concentration approximately 80 nM) source for biomonitoring of exposure to organophosphate anticholinesterases. Polhuijs *et al.* ⁽⁴⁴⁾ developed a procedure for the analysis of phosphorylated binding sites, for example,

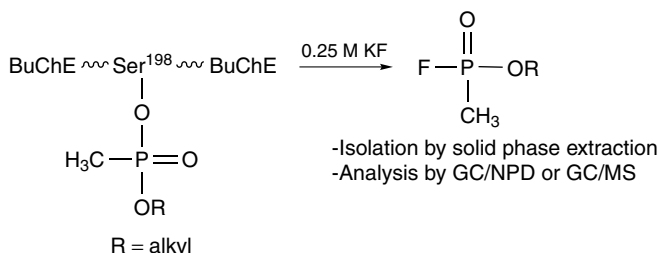


Figure 8. Chemical structures of 2-chlorovinylchloroarsine (Lewisite; L1), 2-chlorovinylarsonous acid (CVAA), British Anti-Lewisite (BAL) and the CVAA-BAL complex. (Reprinted from Toxicology and Applied Pharmacology, Vol. 184, D. Noort, H.P. Benschoop and R.M. Black, Biomonitoring of Exposure to Chemical Warfare Agents: A Review, pages 116–126 (2002), with permission from Elsevier Science.)

BuChE in plasma or serum, based on reactivation of the phosphorylated enzyme with fluoride ions (see Figure 8). This converts the phosphyl moiety quantitatively into the corresponding phosphono- or phosphorofluoridate, which is simply isolated by solid phase extraction on a C_{18} cartridge and quantitated by gas chromatography/nitrogen–phosphorus detection (GC/NPD) or GC/MS. In this way, both the origin and the extent of the organophosphate poisoning can be determined. Furthermore, based on the minimal concentrations of phosphono- or phosphorofluoridate that can be detected in blood, it is calculated that levels $\geq 0.01\%$ inhibited BuChE should be quantifiable, that is, at inhibition levels that are several orders of magnitude less than those that can be measured on the basis of decreased AChE activity. The method is limited by spontaneous reactivation and aging (i.e. loss of the alkyl moiety from the phosphyl group, for instance for soman) of the phosphorylated enzyme and by the natural life span of the enzyme. Recently, the fluoride-reactivation method has been further evaluated for detection of exposure to VX⁽⁴⁵⁾. In this case, the original nerve agent is not regenerated but the analogous phosphonofluoridate.

(c) Hydrolytic displacement of inhibitor from the binding site

An alternative displacement method, reported by Nagao and coworkers^(46,47), is based on a lengthy and more complex procedure. Sarin-bound acetylcholinesterase was solubilized from erythrocyte membranes, digested with trypsin (37°C , 24 h), and the hydrolysis product isopropyl methylphosphonic acid released by digestion with alkaline phosphatase (37°C , 48 h). High molecular mass

materials were removed by ultrafiltration and the aqueous solution concentrated to dryness. Isopropyl methylphosphonic acid was analyzed as its trimethylsilyl derivative by GC/MS.

(d) Mass spectrometric determination of cholinesterase adducts

Recently, Noort and coworkers⁽⁴⁸⁾ developed a versatile procedure that is based on straightforward isolation of adducted BuChE from plasma by means of affinity chromatography with a procainamide column, followed by pepsin digestion and LC/MS/MS analysis of a specific nonapeptide, containing the phosphorylated active site serine-198 residue (see Figure 9). This method surpasses the limitations of the fluoride-reactivation method, since it can also deal with dealkylated (aged) phosphorylated BuChE. Furthermore, the same method could be applied for detection of ChE modifications induced by other inhibitors, for example, diethyl paraoxon and pyridostigmine bromide, illustrating the broad scope of this approach. This new methodology will also allow the biomonitoring of exposure to several OP pesticides and carbamates in one individual, which is highly relevant within the context of the Food Quality Protection Act of 1996. The latter requires the United States Environmental Protection Agency (EPA) to perform a combined risk assessment for chemicals that produce adverse effects by a common mechanism of toxicity (see, for further reading: <http://www.epa.gov/opppsp1/fqpa/>). Within the framework of kinetic and mechanistic studies, comprising the interactions of organophosphates with acetyl- and butyrylcholinesterases, Doorn *et al.*^(49,50) and Elhanany *et al.*⁽⁴⁰⁾ followed a similar approach

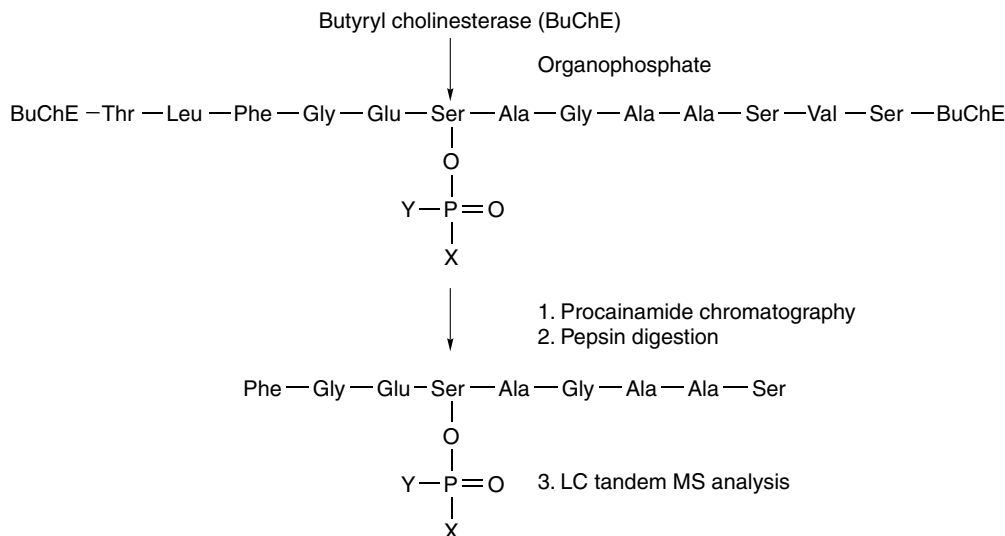


Figure 9. Fluoride reactivation of organophosphate-inhibited butyrylcholinesterase. (Reprinted from Toxicology and Applied Pharmacology, Vol. 184, D. Noort, H.P. Benschop and R.M. Black, Biomonitoring of Exposure to Chemical Warfare Agents: A Review, pages 116–126 (2002), with permission from Elsevier Science.)

by analyzing (modified) peptide fragments in trypsin digests of the cholinesterases with MALDI/TOF/MS.

4.1.3 Application to Human Casualties

All three of the mass spectrometric methods described above were applied to casualties of terrorist attacks in Japan. It should be noted that in most cases the samples were collected within 24 h of the exposure, in contrast to cases of CW exposure to sulfur mustard.

Application of the fluoride-reativation method to serum samples of victims from the Tokyo subway attack, and of the Matsumoto incident, yielded, sarin concentrations in the range of 0.2–4.1 ng/ml serum⁽⁴⁴⁾. Evidently, these casualties had been exposed to an organophosphate with the formula $i\text{-PrO}(\text{CH}_3)\text{P}(\text{O})\text{X}$, presumably with $\text{X} = \text{F}$ (sarin).

The hydrolytic displacement method was applied to four victims killed in the Tokyo subway attack (two died immediately and two later in hospital). Isopropyl methylphosphonic acid was identified at levels sufficient for full scan mass spectra to be obtained using a benchtop quadrupole mass spectrometer⁽⁴⁶⁾. Methylphosphonic acid was also identified. Some two years later, methylphosphonic acid

was detected in formalin-fixed brain tissues from these victims using a similar procedure⁽⁴⁷⁾.

Finally, mass spectrometric determination of the phosphonylated peptic nonapeptide from butyrylcholinesterase allowed the positive identification of sarin-inhibited enzyme in serum samples from several Japanese victims of the Tokyo subway attack (see Figure 10).

4.2 Adduct with a Tyrosine Residue

4.2.1 Site of Reaction

Binding of sarin and soman to a tyrosine residue present in blood has been observed by Black *et al.*⁽⁵¹⁾ When sarin or soman was incubated with human plasma, phosphonylated tyrosine was observed by LC/MS after Pronase digestion, in addition to phosphonylated serine. The precise site of this residue has not yet been confirmed but it is associated with the albumin fraction. A phosphonylated tryptic peptide [$i\text{-PrO}(\text{CH}_3)\text{P}(\text{O})$]-Tyr-Thr-Lys, consistent with albumin, has been identified but this sequence is also present in other proteins. Before the advent of modern mass spectrometry, diisopropyl fluorophosphate was reported to bind

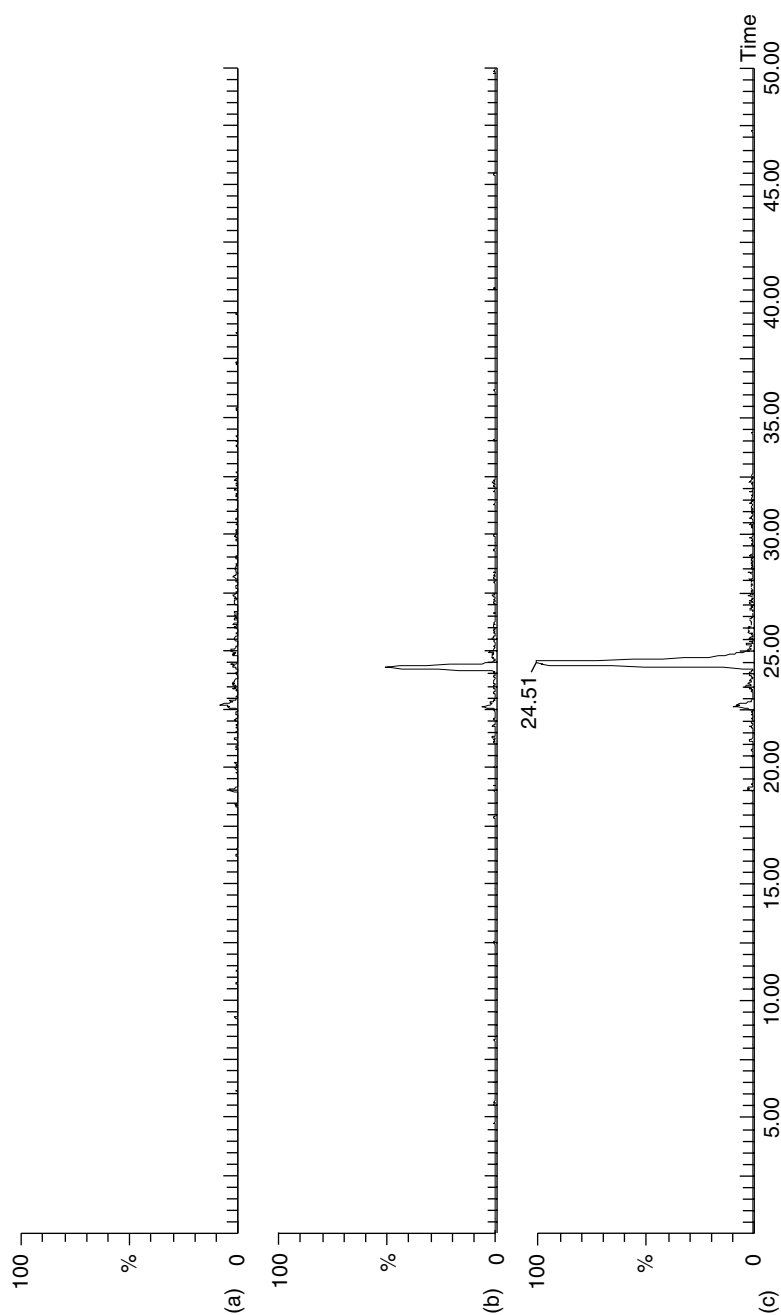


Figure 10. Ion chromatograms of fragment ion m/z 778.4 originating from FGE(S-IMPA)AGAAS, with S-IMPA representing the serine-198 residue conjugated to *O*-isopropyl methylphosphonic acid, in a pepsin digest of human butyrylcholinesterase (HuBuChE). HuBuChE was isolated from serum of nonexposed human blood (a) and from a Japanese victim of the terrorist attack with sarin in the Tokyo metro (b). Trace (c) represents the pepsin digest shown in Trace (a), after spiking with synthetic FGE(S-IMPA)AGAAS (reprinted with permission from Fiddler, A. *et al.*, *Chem. Res. Toxicol.* **15**, 582–590 (2002). Copyright (2002) American Chemical Society)

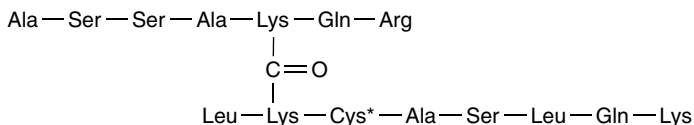


Figure 11. Chemical structure of adduct fragment O=C-(T25–T28) obtained after trypsin digestion of human serum albumin modified at Lys-195 and Lys-199 residues by phosgene

to a tyrosine residue in bovine serum albumin ⁽⁵²⁾. The adduct with soman is formed less abundantly *in vitro* than that for sarin, but in contrast to the adduct with cholinesterase, there is no indication of aging occurring.

4.2.2 Analytical Method

After Pronase digestion of plasma, the analytes were concentrated on a C18 or C8 cartridge and analyzed by LC/MS/MS. The adducts have been detected in the blood of guinea pigs 24 h after being exposed to 0.5 LD₅₀ doses of sarin and soman. It is not known if they are formed in cases of human exposure.

5 PHOSGENE

The pulmonary agent phosgene was used extensively as a chemical weapon in WW I. Nowadays, it is an important intermediate for industrial production of insecticides, isocyanates, plastics, aniline dyes and resins, with an estimated yearly production of almost 1 billion pounds. Reliable diagnosis of exposure to phosgene, other than observation of the developing edema by means of chest roentgenology, is not available.

5.1 Albumin and Hemoglobin Adducts

5.1.1 Sites of Reaction

Noort *et al.* ⁽⁵³⁾ recently demonstrated that phosgene binds effectively to albumin and hemoglobin upon *in vitro* exposure of human blood to [¹⁴C]phosgene ⁽⁵³⁾. Upon Pronase digestion of globin, one of the adducts identified was the pentapeptide O=C-(Val-Leu)-Ser-Phe-Ala, representing amino acids 1–5 of α -globin, with a hydantoin function between the N-terminal valine and leucine. This adduct

did not appear suitable as a biomarker of exposure because a peptide with similar properties was detected at trace levels in control samples, possibly formed by reaction of α -globin with carbon dioxide. *Inter alia*, phosgene appears to crosslink the lysine residues 195 and 199 in human serum albumin (see Figures 11 and 12).

5.1.2 Analytical Method

A method using micro-LC/tandem MS was developed for analysis of the tryptic digest containing the intramolecular albumin lysine–lysine adduct, which enabled the detection of exposure of human blood to $\geq 1 \mu\text{M}$ phosgene *in vitro*. The method has not yet been applied to animal or human samples.

5.2 Adducts to Other Proteins

It has been clearly established that phosgene, as the major metabolite of chloroform, is responsible for the acute toxicity of chloroform ⁽⁵⁴⁾. As part of a study towards the mechanism of the potential carcinogenicity of chloroform, Fabrizi *et al.* ⁽⁵⁵⁾ showed that phosgene was able to form adducts with the N-terminus of human histone H2B, through lysine residues.

6 CONCLUSIONS AND PERSPECTIVES

Adducts with macromolecules, particularly proteins, offer long-lived biological markers of exposure to Chemical Warfare Agent (CWA), possibly up to several months. Gas or liquid chromatography combined with tandem mass spectrometry, are the methods of choice for unequivocal identification of these adducts or metabolites at trace levels. Several

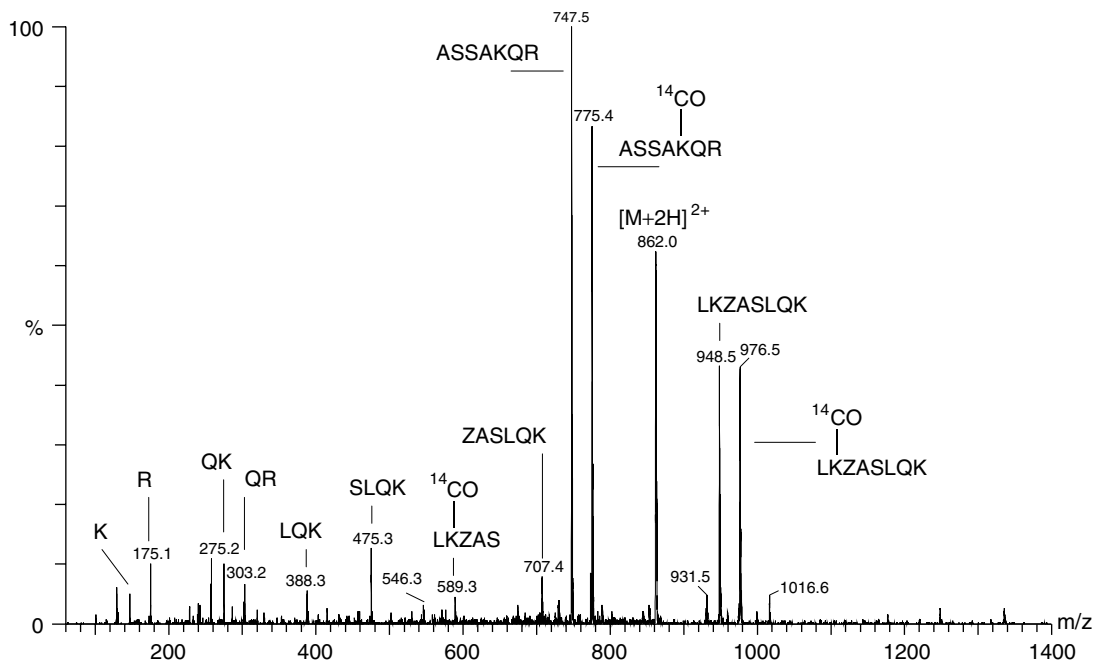


Figure 12. Product ion spectrum of molecular ion $[M + 2H]^{2+}$ (m/z 862.0) of $O=^{14}C$ -(T25-T28) in a tryptic digest of albumin isolated from human blood that was exposed to $[^{14}C]$ phosgene (reprinted with permission from Noort, D. *et al.*, *Chem. Res. Toxicol.*, **13**, 719–726 (2000). Copyright (2000) American Chemical Society

of the developed methods have been applied to actual cases of human exposure to CWA, and have been shown to be highly retrospective.

Many of the methods are highly demanding in terms of detection limits and few of the procedures described are at present easily transferable to routine 'benchtop' mass spectrometer systems. With the exception of ChE inhibition measurements and immunoassays, none of the methods are currently suitable for field laboratories. However, with the rapid ongoing miniaturization of GC/MS and LC/MS equipment, it can be expected that some of the methods will be applicable on-site within a few years. One of the major challenges of researchers in this field is the lowering of detection limits, since exposures to CWA are often limited to single occasions or to very low levels, and experience has shown that samples may become available several weeks or months after the event. In this respect, the tremendous advances in enhancement of the sensitivity and resolution of electrospray mass spectrometry instruments, and several other hybrid configurations, must be recognized.

At the time of the Iraq–Iran conflict, which was the first conflict in modern times where CW was extensively used, no validated procedures were available for biomedical sample analysis. In the following two decades, more than a dozen different methods have been reported for sulfur mustard and nerve agents, although these are currently available in relatively few laboratories. With the rapid progress being made in the identification of new biomarkers of exposure, combined with improvements in instrumentation, it is becoming increasingly likely that significant exposures to a CW agent can be confirmed, provided that appropriate samples are collected.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
AChE	Acetylcholinesterase
BAL	British Anti-Lewisite
CaE	Carboxylesterase
CNS	Central Nervous System
CWA	Chemical Warfare Agent
CVAA	2-chlorovinylarsonous Acid
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
GC/MS	Gas Chromatography/Mass Spectrometry
GC/NPD	Gas Chromatography/Nitrogen-Phosphorus Detection
HPLC	High-Performance Liquid Chromatography
LC/MS/MS	LC/Electrospray Tandem MS
LD ₅₀	50 % Lethal Dose
MALDI/TOF/MS	Matrix-assisted Laser Desorption Ionization/Time-Of-Flight/Mass Spectrometry
PRS	propylsulfonic acid

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